Evaluation on brewery yeast and insect meal (black soldier fly and cricket meal) to replace trash fish in the diet for Asian

Sen Sorphea
Faculty of Veterinary Medicine and Animal Science
Department of Animal Nutrition and Management
Uppsala

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Cover: From top left to right and below from right to left : Fishmeal, brewery yeast, black soldier fly meal and cricket meal. Fish: juvenile Asian seabass

(photo: Sen Sorphea, 2018)

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Errata for
Evaluation on brewery yeast and insect meal (black soldier fly and cricket meal) to replace trash fish in the diet for Asian seabass (Lates calcarifer) in Cambodia

by Sen Sorphea

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Page 34	Location: Line 13 from sub title Is now: fry experiment and 0, 10 psu, 20 psu and 30 Should be: fry experiment and 10 psu, 20 psu and 30
Page 38	Location: Second line in note section Is now: *soldier fly meal. Chemical composition* Should be: *soldier fly meal. †Chemical composition*
Page 39	Location: Line 18 Is now: CrM (147, 300, and 443 g kg^{-1} % Should be: CrM (147, 300, and 443 g kg^{-1})
Page 48	Location: From the bottom line 3

	Is now: respectively (Table 12). Should be: respectively.
Page 50	Location: Table 12 Is now: Daily weight gain (g d^{-1})* Should be: Daily weight gain (g d^{-1})
Page 52	Location: Line 14 from top Is now: the trial period of 2.5 months Should be: the trial period of 2.0 months
Page 53	Location: Table 13 Daily weight gain (g day^{-1}) row 3 Is now: Final Should be: Whole
Page 55	Location: Table 14 at note section part Is now: *Specific growth rate = (Ln Measured weight - Ln Previous measured weight) / No. of days between samplings) x 100;); Survival rate = (Final number of fish / Initial number of fish) x 100;* Should be: *Survival rate = (Final number of fish / Initial number of fish) x 100*
Page 57	Location: Line 6 from top Is now: 80 kg per year Should be: 80 %

Page 40 in the thesis (Table 8)

Is now:

Table 1. *Diet formulation (g kg^{-1}) with inclusion of different levels of brewer's yeast (BY) (Paper III) and black soldier fly meal (BSFM) and cricket meal (CrM) (Paper IV)*

Parameter	Paper III†				Paper IV††						
	BY0	BY1	BY2	BY3	Control	BSFM-1	BSFM-2	BSFM-3	CrM-1	CrM-2	CrM-3
Fish oil	-	-	-	-	50	50	50	50	50	50	50

Should be:

Table 2. *Diet formulation (g kg^{-1}) with inclusion of different levels of brewer's yeast (BY) (Paper III) and black soldier fly meal (BSFM) and cricket meal (CrM) (Paper IV)*

Parameter	Paper III†				Paper IV††						
	BY0	BY1	BY2	BY3	Control	BSFM-1	BSFM-2	BSFM-3	CrM-1	CrM-2	CrM-3
Fish oil	-	-	-	-	50	50	5	30	50	50	50

Page 4 (in paper IV section) (Table 2)

Is now:

Table 2. *Ingredient composition (g kg^{-1} dry matter) of experimental diets with different levels of black soldier fly meal (BSFM) and cricket meal (CrM) replacing fish meal*

Ingredient (%)	Control	BSFM-1	BSFM-2	BSFM-3	CrM-1	CrM-2	CrM-3
Fish oil	50	50	50	50	50	50	50

Should be:

Table 2. *Ingredient composition (g kg^{-1} dry matter) of experimental diets with different levels of black soldier fly meal (BSFM) and cricket meal (CrM) replacing fish meal*

Ingredient (%)	Control	BSFM-1	BSFM-2	BSFM-3	CrM-1	CrM-2	CrM-3
Fish oil	50	50	5	30	50	50	50

Page 54: Table 14

Is now:

Table 3. *Chemical composition (mean ± standard deviation (SD)) of the diets containing different levels of black soldier fly meal (BSFM) and cricket meal (CrM) fed to fish in Paper IV, and of the fish body (analyses performed by National Institute of Animal Sciences, Hanoi, Vietnam)*

Parameter		Control	BSFM-1	BSFM-2	BSFM-3	CrM-1	CrM-2	CrM-3	p-value
Body weight (g)	Initial	12.2 ± 0.82	11.4 ± 1.95	11.6 ± 2.4	11.7 ± 1.35	11.7 ± 0.85	11.8 ± 1.91	11.6 ± 1.87	0.99
	Final	21.5 ± 2.94	26.2 ± 2.31	18.9 ± 3.8	18.4 ± 1.81	21.3 ± 0.23	22.3 ± 5.71	22.7 ± 5.75	0.22
Feed conversion ratio		3.86 ± 0.94^{a}	2.45 ± 0.6^{c}	2.51 ± 0.64^{bc}	2.31 ± 0.41 ^{c}	3.08 ± 1.03^{abc}	3.41 ± 1.69^{ab}	2.40 ± 0.99^{c}	0.001
Specific growth rate (% d^{-1})		0.89 ± 0.52	1.33 ± 0.50	0.78 ± 0.29	0.72 ± 0.22	0.96 ± 0.75	0.99 ± 0.29	1.04 ± 0.54	0.16
Viscero-somatic index (%)		6.48 ± 1.79	6.40 ± 1.14	5.40 ± 0.38	5.91 ± 1.46	5.63 ± 0.76	6.23 ± 0.55	5.85 ± 0.98	0.88
Hepato-somatic index (%)		1.21 ± 0.56	1.52 ± 0.34	1.36 ± 0.35	1.61 ± 0.61	1.39 ± 0.80	1.18 ± 0.26	1.12 ± 0.54	0.88
Survival rate (%)		92.5 ± 12.8	97.1 ± 5.42	93.8 ± 10.3	95.8 ± 7.64	87.1 ± 18.4	92.9 ± 10.8	90.4 ± 15.6	0.50

Should be:

Table 4. *Chemical composition (mean ± standard error (SE)) of the diets containing different levels of black soldier fly meal (BSFM) and cricket meal (CrM) fed to fish in Paper IV, and of the fish body (analyses performed by National Institute of Animal Sciences, Hanoi, Vietnam)*

Parameter		Control	BSFM-1	BSFM-2	BSFM-3	CrM-1	CrM-2	CrM-3	p-value
Body weight (g)	Initial	12.2 ± 0.64	11.4 ± 0.91	11.6 ± 0.91	11.7 ± 0.91	11.7 ± 0.91	11.8 ± 0.91	11.6 ± 0.91	0.99
	Final	21.5 ± 1.47	26.2 ± 2.08	18.9 ± 2.08	18.4 ± 2.08	21.3 ± 2.08	22.3 ± 2.08	22.7 ± 2.08	0.22
Feed conversion ratio		3.72 ± 0.47	2.43 ± 0.64	2.51 ± 0.64	2.30 ± 0.64	4.06 ± 0.68	3.43 ± 0.64	2.40 ± 0.68	0.22
Specific growth rate (% d^{-1})		0.89 ± 0.11	1.33 ± 0.16	0.78 ± 0.16	0.72 ± 0.16	0.96 ± 0.16	0.99 ± 0.16	1.04 ± 0.16	0.16
Viscero-somatic index (%)		6.50 ± 0.47	5.68 ± 0.75	5.82 ± 0.70	6.41 ± 0.71	5.67 ± 0.67	6.12 ± 0.67	5.68 ± 0.67	0.89
Hepato-somatic index (%)		1.21 ± 0.21	1.27 ± 0.33	1.51 ± 0.31	1.79 ± 0.31	1.40 ± 0.29	1.14 ± 0.29	1.06 ± 0.30	0.70
Survival rate (%)		92.5 ± 2.52	97.1 ± 3.56	93.8 ± 3.56	95.8 ± 3.56	87.1 ± 3.56	92.9 ± 3.56	90.4 ± 3.56	0.50

Evaluation on brewery yeast and insect meal (black soldier fly and cricket meal) to replace trash fish in the diet for Asian seabass (*Lates calcarifer*) in Cambodia

Abstract

Sustainable development of marine aquaculture in Cambodia was studied in this thesis, using Asian seabass as the target species. An initial survey mapped current production practices, geographical occurrence and production volumes of Asian seabass farming in coastline provinces in Cambodia (Preah Sihanoukvill, Kampot and Koh Kong). Tolerance to different salinities and alternative local feed sources were then assessed in studies conducted at Marine Aquaculture Research and Development Centre (MARDeC) in Preah Sihanoukvill and at An Giang University, Vietnam. A small digestibility study of all feed sources tested was also conducted at An Giang University.

The survey revealed that fish farming was conducted both in marine and brackish water and that the most common feed used was trash fish. Commercial dry pellets were only used when fish were reared in ponds or for small fish, mainly due to high costs, which were a major constraint preventing farmers from changing from feeding trash fish to pellets.

A series of experiments including two life stages of Asian seabass (fry and fingerlings) and graded levels of salinity found no significant differences in weight gain (WG, g), feed conversion ratio (FCR), daily weight gain (DWG, g day^{-1}), specific growth rate (SGR) or condition factor (CF) in fry or fingerlings at different levels of salinity (fry treatments: 0, 5, 10 and 20 practical salinity units (psu); fingerling treatments 10, 20 and 30 psu).

Experiments on including graded levels of brewer's yeast to replace dietary fishmeal (at 0, 20, 37 and 47 % based on dry matter, denote BY0, BY1, BY2 and BY3), performed in hapa and tanks, indicated only slight, non-significant differences in survival rate (SR, %). Body weight (BW, g) and DWG decreased towards the end of the experimental period, but with no differences between treatments ($p=0.89$ and $p=0.26$). However, the fish tended to display increased feed intake ($p=0.61$ and $p=0.93$) and FCR ($p=0.54$ and $p=0.33$) in hapa and tank respectively with higher level of yeast inclusion, indicating that fish on high levels of yeast need to eat more feed per unit weight gain. In the tank experiment, there was no significant difference in CF or SR, but BW increased around four-fold in all treatments. The main difference between the tank and hapa experiments was in FCR, possibly due to feed losses through the net in the hapa base, while the fish in tanks could feed on the bottom.

A study on using cricket and black soldier fly (BSF) full-fat meal to replace fishmeal in the diet of Asian seabass revealed two major problems: i) The fish were accustomed

to floating pellets and the experimental feed was sinking pellets, which reduced feed intake in all treatments (including control) and resulted in fish weight only doubling. Fish kept on floating commercial pellets in a parallel system performed well, with a four-fold weight increase. A longer adaptation period of the fish to sinking pellets could improve the outcome. ii) The fishmeal used as high-quality protein control was spiked with non-protein nitrogen, indicating that analysis discriminating between protein and non-protein nitrogen should be performed before feed formulation. Overall, the results indicated that both BSF and cricket full-fat meal are potential replacers of fishmeal, and thus also of trash fish, in the diet of Asian seabass. Digestibility analysis confirmed their feed value.

Keywords: Asian seabass, brewer's yeast, cricket, black soldier fly, trash fish,

Author's address: Sen Sorphea,
Marine Aquaculture Research and Development Centre (MARDeC).
Email: sorphea.sen@gmail.com

Dedication

To my dear mother with my respectful gratitude,

My late father with my gratitude,

My aunts, my older brother, older sister,

My younger sisters.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Sen S, Kiessling A, Barnes A C, Da C T, Lindberg J E and Lundh T (2018). A field survey of small-scale cage and pond farming of Asian seabass (*Lates calcarifer*) in Cambodia. *Livestock Research for Rural Development. Volume 30, Article # 130. Retrieved* August 22, 2018, from http://www.lrrd.org/lrrd30/7/torbj30130.html

II Sen S, Terai A, Sreyrum P, Lundh T, Barnes AC, Da CT and Kiessling A (2019). Growth performance of fry and fingerling Asian seabass (*Lates calcarifer*) from Cambodian brood stock reared at different salinities. *Livestock Research for Rural Development. Volume 31, Article #39.* Retrieved April 28, 2019, from http://www.lrrd.org/lrrd31/3/sorph31039.html

III Sen S, Lundh T, Lindberg JE, Da CT, Barnes AC and Kiessling A. Effect of dietary replacement of fishmeal with spent brewer's yeast on growth performance of Asian seabass (*Lates calcarifer*) in Cambodian coastal aquaculture. (Accepted with changes for publication in *Livestock Research for Rural Development*).

IV Sen S, Lan TT, Nguyen HYN, Lundh T, Lindberg JE, Barnes AC and Kiessling A. Replacement of fishmeal by cricket or black soldier fly meal in the diet of Asian seabass (*Lates calcarifer*). (Submitted to *Livestock Research for Rural Development*).

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The contribution of Sen Sorphea to the papers included in this thesis was as follows:

I Participated in planning (preparing questionnaire) for the survey and interviewed farmers, evaluated the results, performed the statistical analyses and wrote the manuscript.

II Participated in planning the experiment, conducted the experiment, collected samples, evaluated the results, performed the statistical analyses and headed the writing process.

III Participated in planning the experiment, conducted the experiment, collected samples, evaluated the results, performed the statistical analyses and headed the writing process.

IV Participated in planning the experiment, carried out feeding and collection of fish and samples, evaluated the results, performed the statistical analyses and headed the writing process.

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Abbreviations

AA	Amino acid
ADC	Apparent digestibility coefficient
BW	Body weight
BSFM	Black soldier fly meal
BSF	Black soldier fly
BY	Brewer's yeast
CP	Crude protein
CF	Crude fat
CL	Crude lipid
CrM	Cricket meal
CMC	Carboxymethyl cellulose
cys+met	Cystine plus methionine
DWG	Daily weight gain
DM	Dry matter
DHA	Docosahexaenoic acid
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
FCR	Feed conversion ratio
FM	Fishmeal
FO	Fish oil
HUFA	Highly unsaturated fatty acid
HSI	Hepato-somatic index
NDF	Neutral detergent fiber
NFE	Nitrogen-free extract
NPN	Non-protein nitrogen
NSP	Non-starch polysaccharides
psu	Practical salinity unit
PUFA	Polyunsaturated fatty acids
RNA	Ribonucleic acid

SGR	Specific growth rate
SR	Survival rate
TL	Total length
VO	Vegetable oil
VSI	Viscero-somatic index
WF	Wheat flour
WG	Weight gain

1 Background

1.1 World aquaculture

World consumption of seafood is increasing and aquaculture will be the most reliable supplier of seafood in coming years, to compensate for the decline in capture fish (Hixson, 2014). Fish is considered a healthy protein source, because it is low in fat and rich in highly unsaturated fatty acids (HUFAs) (Valfré *et al*., 2003), and has particular health benefits for children (Le *et al*., 2009; Dubnov-Raz & Berry, 2007). Fish is also a source of valuable protein and provides a well-balanced amino acid profile for animal and human health that promotes many beneficial effects, including those provided by fish oil (Bell & Waagbø, 2008). For all these reasons, fish consumption has consistently increased and since 2014, the world population has consumed more farmed fish than capture fish (FAO, 2016). According to FAO (2014), this is not only because continuous overfishing of global fish stocks has led to higher prices for captured fish, but also because of an ongoing trend to use pelagic fish directly for human consumption and not mainly for the production of fishmeal.

Fishmeal is the main protein source in aqua feed, with about 3.06 million tons of fishmeal consumed in the aquaculture sector (Abdur *et al*., 2017). Fishmeal is well known as a very digestible feed ingredient in the diet of most farm animals, due to its high energy content and the fact that it is an excellent source of protein, lipids (oil), minerals and vitamins and has a low carbohydrate content (Miles *et al*., 2006). Moreover, the content of digestible protein is above 95%, which allows the feed to be digested rapidly and thereby reduces nutrient leaching, and the content of nutritional inhibitors or anti-nutritional factors is low. Another advantage is that fishmeal contains compounds that make it more acceptable and palatable to fish (Miles *et al*., 2006) and has a good amino acid profile and essential fatty acid content

(Oliva-Teles & Goncalves, 2001). These attributes make fishmeal more valuable than plant protein in aquaculture diets.

1.2 Aquaculture in Cambodia

Cambodia is one of the poorest nations in Southeast Asia, yet has the highest per capita consumption of fish (Vilain *et al*., 2016). Small-scale fishing, recognised as primarily a subsistence activity, is estimated to account for 60% of total inland fisheries production in Cambodia (Joffre *et al*., 2010). Capturing fish from natural water bodies is a seasonal activity, with the peak fishing season starting at the end of the rainy season, although some natural fish stocks appear to have declined over the years. Aquaculture fish production systems in Cambodia are mainly based on inland cage culture, mainly located on the Mekong River (33%), Tonle Sap River (17%), Bassac River (7%) and Tonle Sap Lake (43%). These contribute 70-80% of the country's aquaculture production (So & Haing, 2007; Viseth & Pengbun, 2005). The remaining 20-30% comes from pond-based production systems.

There are conflicting data on fish consumption in Cambodia. Based on a survey, Ahmed *et al*. (1999) concluded that mean per capita consumption of fresh fish is about 49.7 kg year^{-1} for fishing households and 39.9 kg year^{-1} for non-fishing households. Their survey collected data in eight freshwater fisheries provinces (Kompong Chhnang, Pursat, Battamband, Siem Reap, Kandal, Kompong Cham, Kracheh and Stung Treng), including 5117 households and covering 83 communes in 51 fishing districts. With processed fish included, total fish consumption by fishing-dependent communities was estimated at 75.6 kg per capita and year (Ahmed *et al*., 1999). Cambodians are strong consumers of freshwater fish, with annual per capita consumption estimated at 52.4 kg (Hortle, 2007). Other reports (Baran, 2014; IFReDI, 2013; Nam & Leap, 2007) indicate that annual fish consumption in Cambodia is 63 kg per person, accounting for over 76% of total animal protein intake (compared with 20% for pork and beef and 4% for poultry).

In response to projected demand for fish, the Cambodian government has been promoting a range of aquaculture approaches with strong potential for expansion to rural areas of the country. Marine finfish culture has also been strongly promoted in Cambodia, to meet the increasing domestic demand for marine fish. It is very profitable, but requires higher investment in fish seed and feed inputs and more advanced technology than freshwater finfish aquaculture. However, demand is partly met by imported fish from neighbouring Thailand and Vietnam, while domestic production remains limited. Most Cambodian farmers face various technical problems in marine finfish culture, including lack of access to quality fish seed, lack of proper

culture techniques, poor management and disease outbreaks. The government has emphasised the need for more research and development in order to support this growing sector, and some external assistance is being provided by the Southeast Asian Fisheries Development Centre (SEAFDEC) and Japan International Cooperation Agency (JICA). Asian seabass (*Lates calcarifer*) is one of important marine species cultured in Cambodia. Typical practice is to catch wild juveniles or purchase fingerings of groupers (*Epinephelus* sp.), snappers (*Lutjanus malabaricus*), cobia (*Rachycentron canadum*), pompano (*Trachinotus carolinus*) and Asian seabass and fatten them in net cages on feed comprised entirely of marine trash fish.

The aim of this thesis was to help promote sustainable development of marine aquaculture in Cambodia. An initial survey was conducted to collect general information from farmers about seabass culture and feed use in three provinces along the coast (Preah Sihanoukvill, Kampot, Koh Kong). Experiments testing a range of salinity levels were then conducted at the Marine Aquaculture Research and Development Centre (MARDeC) in Preah Sihanoukvill province. In two additional studies, one at MARDeC and one at An Giang University in Vietnam, locally available alternative feed ingredients for seabass (brewer's yeast, cricket meal and black soldier fly meal) were assessed.

1.3 Biology of Asian seabass (*Lates calcarifer*)

Asian seabass, also called barramundi, is one of nine *Lates* species in the family Latidae and is widely distributed in coastal and freshwater regions of the tropical Indo-west Pacific from the Persian Gulf to India to northern Australia (Berra, 2001; Nelson, 1994). Asian seabass is a highly carnivorous and euryhaline fish and is highly fecund, with a single female (>120 cm in total length (TL)) being capable of producing up to 46 million eggs (Ruangpanit, 1986; Davis, 1984; Moore, 1982; Dunstan, 1959). High salinity appears to be an important factor in determining the location of seabass spawning grounds (Davis, 1985; Moore, 1982). These grounds may be located in a variety of habitats, including estuaries, coastal mud flats, headlands and other near-shore waters (Garrett, 1987; Kungvankij *et al*., 1986; Ruangpanit, 1986; Davis, 1985; Moore, 1982). Seabass spawn in association with the monsoon season, with two peaks, during the northeast monsoon (August-October) and the southwest monsoon (February-June) (Ruangpanit, 1986). In the wild, larval seabass retreat into estuarine nursery swamps, where they remain for several months before they return to the estuary or coastal waters (Davis, 1985; Russell & Garrett, 1985, 1983; Moore, 1979). Where the opportunity arises, many juveniles subsequently

move up into the freshwater reaches of coastal rivers and creeks (Davis, 1985; Russell & Garrett, 1985, 1983). Asian seabass are protandrous, *i.e.* the fish mature first as males and become females when they grow older and larger. Juvenile seabass may remain in freshwater habitats until they are 3-4 years of age, when they reach sexual maturity as males, and then move downstream during the breeding season to spawn (Kungvankij *et al*., 1986; Davis, 1982). Some of these fish then change sex at a later stage (4-5 years) and remain female for the rest of their lives (Davis, 1982; Moore, 1979).

Due to fast growth rate, good taste, fleshy texture, high demand and high market value (Robinson *et al*., 2010), Asian seabass is considered a good candidate species for culture in marine, brackish and freshwater environments. Asian seabass is a commercially important farmed species in Australia and Southeast Asia and aquaculture has recently expanded to North America and Europe. Although some Asian seabass are cultured in earthen ponds or sea cages, most are cultured in sea cages located in a river mouth or estuary (Tucker *et al*., 2002; Boonyaratpalin *et al*., 1989). Asian seabass has good market acceptance and high economic values in many countries.

1.3.1 Protein requirement of Asian seabass

The optimal protein content of fish diets has been shown to vary with diet energy content and fish size (Boonyaratpalin, 1997; Catacutan & Coloso, 1995). Most studies suggest a protein requirement of 45-55% in the diet. For juveniles, a protein to energy ratio of 25-30 g MJ^{-1} is suggested. Studies on dietary lipid requirements, for energy and essential fatty acids (EFA), show that smaller fish perform best with a dietary lipid content of 14-16%, while growth of larger fish continues to improve with a lipid content up to 19%, with fish oil being the major dietary lipid (Glencross, 2011a; Carter *et al*., 2010; Boonyaratpalin & Williams, 2002). The natural foods of Asian seabass are high in protein, so it can be assumed that the fish do not utilise carbohydrates well. It has been suggested that the carbohydrate content in the feed for Asian seabass should between 10 and 20 % (Boonyaratpalin, 1988). The vitamin requirement depends on the size, stage of sexual maturity, growth rate, environmental conditions and dietary nutrient interrelations, and seems to decrease as fish size increases, *e.g*. Boonyaratpalin *et al*. (1989a, 1989b) observed the best growth rates when fish were fed diets containing 500-1000 mg vitamin C kg^{-1} diet or higher. A dietary level of 30 mg ascorbyl-2 monophosphate kg^{-1} diet or 25 mg ascorbic acid-glucose kg^{-1} diet (equivalent to 12.6 mg or 13 mg ascorbic acid kg^{-1} diet, respectively) is reported to result in normal growth and prevent deficiency signs (Wanakowat *et al*., 1989). When this diet was used to determine the pyridoxine

requirement, the results indicated that 5 mg of pyridoxine kg^{-1} of diet was required for normal growth and 10 mg of pyridoxine kg^{-1} of diet was needed for normal lymphocyte levels (Wanakowat *et al*., 1989). Energy demand is less than 500 g kg^{-1} live weight (Glencross, 2006). The requirement of the amino acid tryptophan is around 0.5% of dietary protein (Coloso *et al.,* 1993), while that of methionine, lysine and arginine has been determined to be around 2.2%. 4.9% and 3.8%, respectively (Millamena *et al*., 1994). The optimal level of n-3 highly unsaturated fatty acid (HUFA; EPA+DHA) in the diet of marine fish larvae is around 3% of dry matter (Zambonino Infante & Cahu, 1999). Glencross *et al*. (2013) estimated the dietary phosphorus (P) requirements for juvenile Asian seabass to be 0.55-0.65% of diet dry matter. Protein requirement data from different studies are summarised in Table 1, Summary of vitamin deficiencies (Table 2).

Table 1. *Summary of protein requirement estimates for Asian seabass (Lates calcarifer)*

Crude protein levels examined (%) (max-min)	Optimal level of crude protein (%)	Gross energy level at optimal (MJ kg^{-1})	Initial fish size (g)	Temperature (^{0}C)	Source
35 - 55	45 - 55	13.4 - 16.4	n/d	n/d	Cuzon & Fuchs (1988)
45 - 55	50	n/d	7.5	n/d	Sakaras *et al*. (1988)
45 - 55	45	n/d	n/d	n/d	Sakaras *et al*. (1989)
35 - 50	50	50	1.3	29	Catacutan & Coloso (1995)
29 - 55	46 - 55	18.4 - 18.7	76	28	Williams & Barlow (1999)
38 - 52	52	17.8 - 21.0	230	28	Williams *et al*. (2003)
44 - 65	60	20.9 - 22.8	80	28	Williams *et al*. (2003)

n/d = no data.

1.3.2 Feed sources for Asian seabass

There is clear potential to reduce the fishmeal content of Asia seabass diets to as little as 150 g kg^{-1} without loss of productivity (Glencross *et al*., 2011b). The growth of larger (70 g average initial weight) Asian seabass over six weeks is reported to be doubled by complete replacement of fishmeal with lupin protein concentrate, lupin kernel meal, wheat gluten, rapeseed meal or poultry offal meal. Glencross *et al*. (2016) used poultry meal, soybean meal and rice bran oil to partly or completely replace both fishmeal and fish oil in

Asian seabass diets (155 g average initial weight) and found that, after eight weeks, weight gain was significantly affected by fishmeal replacement, but not by the level of fish oil inclusion. Those authors concluded that fish oil could be completely replaced with vegetable oil and that up to 70-90% of fishmeal in the diet could be replaced without causing growth performance problems (Glencross *et al*., 2016).

Table 2. *Summary of vitamin deficiencies for Asian seabass (Lates calcarifer). Source: Boonyaratpalin and Wanakowat (1993a 1993b)*

Vitamin	Requirement (mg kg^{-1} diet)	Deficiency signs
Thiamine	R	Poor growth, high mortality, stress susceptible, substantial post-handling shock
Riboflavin	R	Erratic swimming, cataracts
Pyridoxine	5-10 [a]	Erratic swimming, high mortality, convulsions, avoidance of schooling
Pantothenic acid	15 – 90 [a]	High mortality, ventral fin haemorrhage and erosion, haemorrhage acid around operculum and isthmus, mortality in 5-6 weeks
Nicotinic acid	n/d	Fin haemorrhaging and erosion, clubbed gills, high mortality
Biotin	n/d	
Inositol	R	Poor growth, abnormal bone formation
Choline	n/d	
Folic acid	n/d	
Ascorbic acid (Vitamin C)	25 – 30 [b] (700 [c])	Gill haemorrhages, exophthalmia, scoliosis, lordosis, broken back syndrome
		Fatty liver, muscle degeneration, poor gill development, bone deformations, distortion of gill filament and hyperplasia, short operculum, short snout, exophthalmia, short body, loss of equilibrium, scoliosis, lordosis, pop-eye, low blood parameters and low tissue hydroxyproline
Vitamin A	n/d	
Vitamin D	n/d	
Vitamin E	R	Muscular atrophy, increased disease susceptibility
Vitamin K	n/d	

[a]For maximum tissue storage; [b]For ascorbyl-2-monophosphate-Mg or ascorbic acid glucose.

[c]For crystalline vitamin C; R = Required, but quantity required not estimated, n/d = no data available.

1.4 Brewer's yeast

Spent brewer's yeast is the second major by-product from the brewing industry. It is low in calories, fat and carbohydrates, and it can be a valuable source of cheap fibre, mainly ß-glucans (Martins *et al*., 2015; Aimanianda *et al*., 2009; Liu *et al*., 2008), nucleotides (Vieira *et al*., 2013), vitamins and minerals (Ferreira *et al*., 2010) and chitin (Siwicki *et al*., 1994). The European Food Safety Authority (EFSA) has already approved the use of *Saccharomyces* ß-glucans, referred to as "yeast beta-glucans", as a new food ingredient and suggests a dose ranging between 50 mg and 200 mg per serving (EFSA, 2011). ß-glucans are structural components of the cell wall of bacteria, fungi, yeasts and some plants. In recent years, the effective immunomodulatory properties of ß-1,3/1,6-glucan derived from yeast have been extensively proven, not only in mammals but also in fish (Soltanian *et al*., 2009; Volman *et al*., 2003). ß-glucans naturally form polysaccharides, with glucose molecules linked by ß-glycosidic bonds (Tokunaka *et al*., 2000), and can stimulate macrophages to actively fight against fish pathogens (Cook *et al*., 2003). Dietary nucleotides reduce stress responses, including cortisol release, in rainbow trout (*Oncorhyncus mykiss*) (Leonardi *et al*., 2003).

1.4.1 Brewer's yeast utilisation in fish

Several studies report that replacement or supplement of brewer's yeast (*Saccharomyces cerevisiae*) can enhance immune responses and/or disease resistance in a number of fish species such as: rainbow trout (Siwicki *et al*., 1994), gilthead sea bream (*Sparus aurata L.*) (Ortuño *et al*., 2002), hybrid striped bass (*Morone chrysops* × *M. saxatilis*) (Li & Gatlin, 2004), Atlantic salmon (*Salmo salar*) (Engstad *et al*., 1992), European seabass (*Dicentrarchus labrax*) (Oliva-Teles & Goncalves, 2001), Asian catfish (*Clarias batrachus*) (Kumari & Sahoo, 2006) and pacu (*Piaractus mesopotamicus*) (Ozório *et al*., 2010). Robertsen (1999) found that activation of the immune system mechanism by the yeast cell wall of ß-glucan stimulated the phagocytic function and increased protection in several fish species following challenge with pathogenic bacteria. Pongpet *et al.* (2016) found that the brewer's yeast could replace up to 45% of fishmeal with improved growth performance of Thai panga (*Pangasianodon hypophthalmus* x *Pangasius bocourti*). These positive effects of brewer's yeast might be due to stimulation of extracellular enzyme secretion by the gut microflora, which increases digestion and absorption of the diet. However, increasing the level up to 60-75% of brewer's yeast results in a significant decrease in growth performance, possibly due to poor utilisation of non-

starch polysaccharides (NSP), an indigestible carbohydrate contained in plant feed ingredients and brewer's yeast (Pongpet *et al*., 2016). Ebrahim *et al*. (2008) examined the possibility of replacing an expensive protein source (fishmeal) with a less expensive source (brewer's yeast) in the diet of Nile tilapia (*Oreochromis niloticus*) and found that the fish grew better when fed diets containing yeast/fishmeal at a rate of 50/50 and 75/25 (%) than when fed diets containing yeast or fishmeal alone. Oliva-Teles and Gonçalves (2001) report that brewer's yeast can replace up to 50% of fishmeal protein with no negative effects on the performance of juvenile European seabass with an initial average weight of 12 g.

1.5 Why insect meal?

Insects are the largest community of organisms in the global ecosystem. Exploration and use of insect resources as a protein source in the animal feed industry can be an important component of sustainable and ecologically sound development (Sánchez-Muros *et al*., 2014). Compared with conventional animal protein, insects have several advantages, including that they can be reared on discarded organic by-products with low water inputs, less land area, high feed conversion efficiency, low emissions of greenhouse gases and ammonia, few animal welfare issues and a low risk of transmitting zoonotic infections (van Huis *et al*., 2013; Oonincx *et al.*, 2010).

Insects are also good sources of minerals such as potassium (K), calcium (Ca), iron (Fe), magnesium (Mg) and selenium (Se) and of several vitamins, the levels of which depend on the rearing conditions (Henry *et al*., 2015). Once processed, the original waste product becomes insect biomass and insect frass; the larvae are a value-added product and the frass is much easier to manage than the original wastes (Diener *et al*., 2011).

This thesis examined the fish feed potential of two widely available insects in Cambodia (black soldier fly and crickets). Crickets are popular as food in Cambodia and the market has been described as fast growing (Miech *et al*., 2016; Münke, 2012). Work by Bondari and Sheppard (1981) has revealed the possibility of using soldier fly larvae in commercial fish production. Crickets are also believed to be potential sources of nutrients for fish (Makkar *et al.*, 2014). In general, compared with conventional livestock, insects have higher feed conversion efficiency (Nakagaki & Defoliart, 1991), *i.e.* they need less feed for production of 1 kg biomass, and have higher fecundity (*e.g*. the common house cricket lays up to 1500 eggs over a period of about a month). Both black soldier fly and cricket are mostly omnivorous and can be raised on organic waste, are equally nutritious and take up little space in the rearing process.

1.5.1 Black soldier fly (*Hermetia illucens*)

Black soldier fly (*Hermetia illucens*), a member (Diptera) of the Stratiomyidae family, was first recorded at the Hilo Sugar Company in the Hawaiian Islands in 1930. The tropical, subtropical and warm temperate areas of America are its native areas. Black soldier fly (BSF) has since transferred to many regions around the world (Leclercq, 1997), and it is now widespread in tropical and warmer temperate regions (Diener et al., 2011). The inadvertent transmission of disease is not a concern with BSF, as it is not a vector for human pathogens. In fact, BSF can limit housefly populations through competitive exclusion under natural conditions in the field (Bradley & Sheppard, 1984; Sheppard, 1983). The species can also help reduce infectious bacterial populations in chicken manure and cow manure (Liu et al., 2008; Erickson et al., 2004).) Female BSF lay about 1000 eggs in their 5-8 day lifespan (Figure 1) and prefer to lay their eggs in cracks and crevices near or above decaying matter, such as carrion, dung, rubbish and other organic waste, thus allowing the eggs to have a better chance of survival.

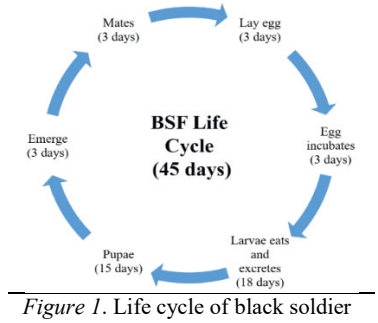

Figure 1. Life cycle of black soldier fly (BSF).

The use of BSF larvae as a feedstuff has been investigated by several research groups, *e.g.* for chickens (Hale, 1973), pigs (Newton *et al*., 1977), channel catfish and blue tilapia (Bondari & Sheppard, 1987), broiler chickens (Elwert *et al*., 2010) and turbot (Kröckel *et al*., 2012). Mostly positive results have been obtained and it has also been shown that BSF larvae meal can provide an amino acid pattern comparable to that of fishmeal (Elwert *et al*., 2010).

1.5.2 Chemical composition of BSF

Black soldier fly larvae are nutritionally very rich (Tables 3-5), containing on a dry matter basis 40-45% protein, 30-35% fat, 11-15% ash, 4.8-5.1% calcium and 0.6% phosphorus, as well as a range of amino acids and minerals (Yu & Chen, 2009). However, estimates of the amount of fat in BSF larvae are extremely variable and the value depends on the type of diet, *e.g.* 15-25%

in larvae fed on poultry manure (Arango Gutierrez *et al*., 2004), 28% in larvae fed on swine manure (Newton et al., 2005), 35% in larvae fed on cattle manure (Newton *et al*., 1977) and 42-49% in larvae fed on oil-rich food waste (Barry, 2004). The larvae tend to contain less crude protein (CP) and more lipids than those of housefly (*Musca domestica*). The ash content is relatively high but the values reported are highly variable (range 11-28% dry matter (DM)), and BSF larvae are rich in calcium (up to 8% DM) and phosphorus (up to 1.5% DM) (St-Hilaire *et al*., 2007b; Arango Gutierrez *et al*., 2004; Newton *et al*., 1977). The lysine content is particularly high (6-8% of CP). The dry matter content of fresh BSF larvae is quite high, in the range 35-45%, which makes them easier and less costly to dehydrate than other fresh by-products (Newton *et al*., 2008).

Table 3. *Chemical composition of black soldier fly larvae*[†]

Crude protein (% in dry matter (DM))	42.1
Crude fibre (% in DM)	7.0
Ether extract (% in DM),	26.0
Ash (% in DM)	20.6
Gross energy (MJ/kg DM)	22.1
Mineral content (values in g/kg DM except Mn, Zn and Cu, which are in mg/kg DM)[††]	
Calcium (Ca)	75.6
Phosphorus (P)	9.0
Potassium (K)	6.9
Sodium (Na)	1.3
Magnesium (Mg)	3.9
Iron (Fe)	1.37
Manganese (Mn)	246.0
Zinc (Zn)	108.0
Copper (Cu)	6.0

[†]*Sources: Arango Gutierrez et al. (2004) and Newton et al. (1977).*

[††]*Source: Arango Gutierrez et al. (2004), Newton et al. (1977) and St-Hilaire et al. (2007a,b).*

The fatty acid composition of BSF larvae depends on the fatty acid composition of the diet (Table 4). The lipids of larvae fed on cow manure contain 21% lauric acid, 16% palmitic acid, 32% oleic acid and 0.2% omega-3 fatty acids, while the corresponding proportions for larvae fed 50% fish offal and 50% cow manure are 43%, 11%, 12% and 3%, respectively. Total lipid content can also increase, from 21% to 30% DM. In a recent study, oil from BSF larvae was also used to produce biodiesel (Zheng *et al*., 2013). As shown in Tables 4 and 5, the amount of protein in insect bodies varies

according to the stage of development (larva, pupa, prepupa, imago), the type of diet and the rearing conditions. As a consequence of these variations, the amino acid content can also vary, as shown by Finke (2002) (Table 4). However, it has been shown that the fatty acid composition of BSF is highly dependent on the fatty acid composition of the feed substrate (Oonincx *et al.*, 2015; St-Hilaire *et al.*, 2007b).

Table 4. *Protein and lipid content of black soldier fly (Hemetia illucens), express in g kg^{-1} per sample depending on growth stage and type of food substrate*

Life stage	Type of food received	Composition		Authors	
		Dry matter	Protein	Lipid	
Larvae	Chicken feed	387.0	412.0	3360	Spranghers *et al*. (2017)
Larvae	Vegetable waste	410.0	399.0	371.0	Devic *et al*., (2018)
Larvae	Restaurant waste	381.0	431.0	386.0	
Larvae	Brewery solid waste, water, wheat bran, yeast slurry, processing wastes from fish feed factory	950.3	416.4	234.4	
Larvae	Fruit waste	282.9	307.5	407.0	Meneguz *et al*. (2018)
Larvae	Vegetable-fruit waste	219.6	418.8	262.8	
Larvae	Brewery by-product	290.8	529.6	298.7	Xiao *et al*. (2018)
Larvae	Fresh chicken manure		470.0	177.0	
Prepupae	Swine manure	916.0	436.0	331.0	St-Hilaire *et al*. (2007a)

Table 5. *Proximate composition of black soldier fly pre-pupae reared on different substrate*

Parameter	Chicken feed	Digestate	Vegetable waste	Restaurant waste
Moisture, g kg^{-1}	613	614	590	619
Crude protein, g kg^{-1} DM	412	422	399	431
Chitin, g kg^{-1} DM	62	56	57	67
Ether extract, g kg^{-1} DM	336	218	371	386
Crude ash, g kg^{-1} DM	100	197	96	27

Source: Spranghers *et al*. (2017).

1.5.3 Black soldier fly larvae in fish feed

Studies in which 10% fishmeal is replaced with BSF meal in the diet of different fish species such blue tilapia (*Oreochromis aureus*) and channel catfish (*Ictalurus punctatus*) show slow growth rate (Bondari & Sheppard, 1981). In yellow catfish (*Pelteobagrus fulvidraco*), 25% replacement of fishmeal by BSF larva powder has been found to produce no significant difference in growth index and immunity index compared with those in a control group (Zhang *et al*., 2014a,b). In rainbow trout, one study found that BSF meal made from pre-pupae reared on dairy cattle manure enriched with 25-50% trout offal could be used to replace up to 50% of fishmeal protein in the diet for eight weeks without significantly affecting fish growth or the sensory quality of trout fillets, although a slight (but non-significant) reduction in growth was observed (Sealey *et al*., 2011). In another nine-week study, replacing 25% of fishmeal protein in rainbow trout diets with BSF pre-pupae meal (reared on pig manure) did not affect weight gain and feed conversion ratio (St-Hilaire *et al*., 2007a). In a study on Atlantic salmon in which BSF larvae replaced 25%, 50% or 100% of fishmeal in a control diet containing 200 g/kg, histological examinations did not reveal differences between any of the dietary groups and sensory testing of fillets did not reveal any significant differences (Lock *et al*., 2014). Juvenile turbot (*Psetta maxima*) accepted diets containing 33% defatted BSF larvae meal (as a replacement for fishmeal) without significantly affecting feed intake and feed conversion (Kroeckel *et al*., 2012). However, specific growth rate was lower at all inclusion rates and higher inclusion rates decreased acceptance of the diet, which resulted in reduced feed intake and lower growth performance. The presence of chitin might have reduced feed intake and nutrient availability and therefore reduced growth performance and nutrient utilisation (Kroeckel *et al*., 2012). Table 5 summarises data from Spranghers *et al*. (2017) on the chemical composition and chitin content of BSF larvae reared on different substrates. In European seabass, BSF can be included successfully up to 19.5% in the diet (corresponding to 22.5% of total dietary protein) without negative effects on growth and fish performance (Sánchez López *et al*., 2016).

1.5.4 Crickets

Crickets are a common food in Southeast Asia. The house cricket (*Acheta domestica*), the field cricket (*Gryllus testaceus*), *Gryllus bimaculatus*, *Teleogryllus occipitalis*, *Teleogryllus mitratus*, the short-tail cricket (*Brachytrupes portentosus*) and *Tarbinskiellus portentosus* are all edible cricket species (van Huis *et al*., 2013). Cricket is also good protein source,

since it can grow on some substrates that are not suitable for human consumption, such as cassava leaves. Caparros Megido *et al*. (2016) concluded that young cassava leaves and brown rice (with or without bananas) can be used in cricket diets and produce crickets with a high total biomass, while diets made of taro aerial parts or only young cassava leaves can be used to produce crickets with a high protein level. The field cricket's eggs are usually laid in the soil. The newly hatched nymphs burrow to the surface and begin to feed on a variety of succulent grasses and weeds. The nymphs look like the adults except for their smaller size and the absence of wings. The nymphs moult 8 to 10 times over a period of 2-3 months before becoming adults. In field crickets, the chitin content is 8.7% (Wang *et al*., 2004). The house cricket is easy to farm and can produce 6-7 generations per year. It is omnivorous and can eat a large range of organic materials. Production is feasible at temperatures higher than 20 °C and the ideal temperature is 28-30 °C. Approximately 2000 insects can be bred in an area of 1 m^2. Cricket populations are self-regulated by cannibalism (Hardouin & Mahoux, 2003).

1.5.5 Chemical composition of crickets

Adult crickets contain up to 22% neutral detergent fibre (NDF), compared with 12% for the nymphs (Finke, 2002). The crude protein content of house cricket is also very high (55-67%) (Table 6). The calcium and phosphorus contents in house cricket are both higher than in locust or grasshopper meal, while the lysine and cystine plus methionine (cys+met) contents are lower than in locust meal. The palmitoleic acid level in house cricket is approximately 15-fold lower than that in housefly maggots and 4-fold lower than that in mealworm. On the other hand, the level of linoleic acid is higher in house cricket. As in other insects, the crude protein content of field cricket and Mormon cricket (*Anabrus simplex*) is high (ca 60%) and they contain 10-13% lipids. The calcium content in Mormon cricket is low (2 mg kg^{-1} DM). The lysine content is lower in field cricket than in Mormon cricket, while the level of sulphur-containing amino acids (cys+met) is higher in field cricket.

Table 6. *Chemical composition of two cricket species*

	House cricket†	Field cricket††
Crude protein (% of dry matter (DM))	63.3	58.1
Neutral detergent fibre (% in DM)	18.3	-
Acid detergent fibre	10.0	-
Ether extract DM)	17.3	10.3
Ash (% in DM)	5.6	3
Gross energy (MJ/kg DM)	-	23.0
Mineral content of house cricket (all values in g/kg DM except for Cu, Mn, Fe and Zn, which are in mg/kg DM.		
Calcium (Ca)	10.1	-
Phosphorus (P)	7.9	-
Magnesium (Mg)	1.2	-
Copper (Cu)	15.0	-
Manganese (Mn)	40.0	-
116.0Iron (Fe)	116.0	-
Zinc (Zn)	215.0	-

Sources: †Barker *et al*. (1998) and Finke (2002).
††DeFoliart *et al*. (1982), Nakagaki *et al*. (1987) and Wang *et al*. (2005).

1.5.6 Cricket meal in fish feed

In a study by Taufek *et al*. (2018), various inclusion percentages of field cricket meal (0% (control), 25%, 50%, 75% and 100%) were formulated to yield a diet with an isonitrogenous content of 28% crude protein. The results showed that a practical diet containing 100% cricket meal is appropriate for growth and nutrition utilisation in African catfish (*Clarias gariepinus*) fingerlings. The data obtained by Taufek *et al.* (2016a) during that experiment suggested that dietary cricket meal could improve growth performance of African catfish and enhance feed efficiency. Moreover, the haematological findings supported the fact that a diet containing up to 100% cricket meal is suitable for feeding African catfish, without adverse effects.

2 Objectives of the thesis

Overall aim

To promote sustainable development of marine aquaculture in Cambodia.

Specific objectives

- To perform a survey in order to understand present production practices, geographical occurrence and production volumes of Asian seabass farming in coastal provinces of Cambodia (Paper I)

- To evaluate the effects of different water salinity levels on growth and other production variables in Asian seabass (Paper II)

- To evaluate brewer's yeast as an affordable and functional local alternative to trash fish in feed to Asian seabass (Paper III)

- To evaluate low-technology feed protein alternatives to trash fish in the diet of Asian seabass, in the form of locally available insects that can be produced on local waste streams (Paper IV).

- To evaluate the digestibility in Asian seabass of the feed sources used in Papers III and IV, allowing for more precise feed formulation (this thesis).

3 Materials and methods

This chapter briefly summarises the material and methods used in the studies described in Papers I-IV. It also provides a description of the small additional study of digestibility. A survey (Paper I) and three growth studies were performed. One of the growth studies was at different salinities (Paper II) and two studies tested the alternative protein sources brewer's yeast (Paper III) and black soldier fly larvae meal and cricket meal (Paper IV). A randomised design was employed in all experiments.

3.1 The survey

The survey was conducted in three provinces (Preah Sihanoukvill, Kampot and Koh Kong) along the coast in Cambodia, in order to assess the current situation in Asia seabass farming (small and medium scale). A total of 56 households were interviewed, with 20, 11 and 25 farmers in Preah Sihanoukvill, Kampot and Koh Kong, respectively. Primary data were obtained from the local fishery authority. Only farmers who culture Asian seabass (in monoculture or in polyculture with other marine species) were selected for interview.

3.1.1 Interviews

The farmers were interviewed using a structured questionnaire to collect information on: (i) general characteristics of the fish culture, socio-economic characteristics (the fish farmers' education level, experience of fish farming, other occupation of farmers *etc*.), (ii) details of fish culture practices (number of cages/ponds, source of fish seed, stocking density, technical resources, water sources, mortality, common fish diseases (symptoms), cause and effect of diseases, opportunities and constraints (problems of disease, water pollution), number of workers employed *etc*., (iii) type of feed used and feeding regime and (iv) size or weight at harvest and fish yield for the last calendar year.

3.2 Fish experiments

3.2.1 Facilities and fish rearing conditions

The studies described in Papers II and III were conducted at the Marine Aquaculture Research and Development Centre (MARDeC), which is located in Preah Sihanoukvill province in Cambodia. Asian seabass fry and fingerlings produced at MARDeC from local brood-stock were used in growth performance experiments on larvae and fingerlings at different water salinities (Paper II). Fingerlings were used to evaluate the effect of replacement of fishmeal with brewer's yeast (Paper III).

In Paper II, two separate experiments were conducted, one with fry (14-day study period) and the other with fingerlings (54-day study period). Four different salinity levels were tested in duplicate. These were: 0 (freshwater), 5 psu, 10 psu and 20 psu for the fry experiment and 0, 10 psu, 20 psu and 30 psu for the fingerling experiment. The salinity experiment (Paper II) was run in duplicate and triplicate in 200-L circular tanks (650 mm deep x 750 mm high) connected to individual canister-type biofilters with continuous aeration supply, via a central compressor airline for fry and fingerlings. The stocking density was 100 fry (total 800 fry) at 42 days old with an average size of 0.3 g (TL 21-25 mm) in the fry trial and 10 fingerlings (total 90 fingerlings, equivalent to 50 fingerlings per m^3) at an average weight of 6-7 g in the fingerling trial.

Two separate experiments were also performed in Paper III, one using hapas and one using separate fish tanks with a volume of 1 m^3. The experiments were conducted at different times of the year, since the tank facility was not available all the time, and the intention was not to make any direct comparison between those two systems. However, the same feed formulation containing brewer's spent yeast to replace fishmeal was used. A total of 16 hapa nets (1.5 m length x 1 m width x 1.5 m depth), equipped with individual air stones, were immersed for 60 days in two identical concrete tanks, each with volume 42 m^3 (8 hapas per tank). Seawater flow through the system (30 psu salinity) was maintained, with a complete water change every 24 hours. Asian seabass fingerlings (1120, average weight 40-44g) produced at MARDeC were stocked at 70 fingerlings per hapa net and allocated to one of four treatments, with four replicates per treatment, randomly distributed between the two tanks.

In the tank experiment, 12 plastic tanks containing 1 m^3 of water were used, with three replicates per treatment. A total of 540 Asian seabass fingerlings at weight ranging from 22-23 g were divided between the tanks,

with 45 fingerlings per tank. The tank environment allowed the fish to feed from the bottom. The tank experiment was also run for 60 days, with a water flow rate into the tank of 0.2 L min^{-1} using the same seawater supply as in the hapas experiment. The tanks were equipped with both a central and an external standing pipe, to allow flushing of the tanks twice daily, at 1 h post feeding.

The study in Paper IV was conducted at An Giang University, Vietnam, from May to August 2018 (9 weeks). Two full-fat protein sources from insects, namely cricket meal (CrM) and black soldier fly meal (BSFM) were used to replace fishmeal and plant oil in the diet of Asian seabass. The experiment had a complete randomised design with total of eight treatments, including an extra commercial dry pellet feed, with three replicates per treatment. The purpose of including a commercial feed was as an indicator of whether the environment in the experiment would cause any stress to the experimental fish. A total of 27 composite settlement tanks (allowed to collect the remaining feed and flush out the faeces after and before feeding) with capacity 500 L per tank were used in this experiment (including those in the control treatment). A total of 540 fingerlings at an average size of 8-10 g, brought from Nha Trang province in Vietnam, were stocked at 20 fingerlings in each individual tank with culture water of salinity 10 psu.

3.2.2 Experimental diets

In Paper II, a commercial diet was used to feed the fry for 14 days. They were fed by hand twice a day, at 08:00 h and 17:00 h, at 10% biomass per day, with a feed size of 500-800 µm and a protein content of 55% (INVE Thailand Company Ltd.). Uneaten feed was collected after each feeding by siphon and water was replaced as necessary with water of identical salinity from a pre-prepared stock. The fingerlings were fed to satiation with a commercial dry pellet feed, Ocialis C1 (Ocialis-BERNAQUA ASIA, Ho Chi Minh, Vietnam), for 56 days. The proximate composition (provided by the manufacturer) was moisture 14%, crude protein 58% (min), digestible protein 55% (min), metabolisable energy 5000 kcal/kg (min), crude fibre 1%, calcium 1.5-2.5%, lysine 3.2% and cys+met 2% (min). Fish sampling was carried out every 14 days.

Before formulating the diets (Papers III and IV), the ingredients were analysed for gross chemical composition, crude fibre, ash, energy and amino acids (Table 7). Dry brewer's yeast was obtained as beer-making residues from the Angkor Beer company in Preah Sihanoukvill province. Four experimental diets were formulated (Paper III), to comprise 45% protein and

14% lipid, with brewer's yeast (BY) replacing fishmeal at a level of 0, 20, 37 and 47 % (denoted BY0, BY1, BY2 and BY3, respectively).

Table 7. *Chemical composition (% of dry mater (DM)) of the ingredients and metabolisable energy (ME, MJ kg^{-1}) in the diet used in Paper III, gross energy (GE, MJ kg^{-1}) in the diet in Paper IV and amino acid (% of DM) content of feed ingredients*

Parameter	Paper III†				Paper IV††				
	FM	SBM	BY	WF	FM	SBM	CrM	BSFM	WF
Dry matter, DM	91.8	91.3	93.6	88.6	91.4	88.1	97.2	87.1	85.1
Crude protein	62.2	46.2	46.8	11.6	68.6	57.3	59.2	40.2	14.5
Crude fat	6.4	5.8	1.7	5.7	7.39	1.11	22.0	31.3	0.16
Crude fibre	4.6	3.0	2.2	0.8	4.96	2.44	9.66	9.51	0.16
Ash	2.2	6.4	5.6	0.9	18.9	7.25	5.38	9.47	0.66
ME† , GE††	10.5	17.3	16.0	17.3	16.4	19.1	24.9	25.5	16.9
Essential amino acids									
Arginine	2.40	2.12	1.42	0.35	1.70	3.06	3.23	2.53	0.32
Histidine	1.07	0.51	0.43	0.12	0.34	0.72	0.80	0.59	0.11
Isoleucine	2.23	2.03	1.51	0.52	1.37	2.29	2.46	1.86	0.33
Lysine	4.32	2.94	2.74	0.34	1.03	3.34	3.37	2.53	0.35
Leucine	3.61	2.55	2.03	0.64	2.22	3.34	3.65	2.70	0.66
Methionine	1.43	0.97	0.73	0.18	0.53	0.64	1.14	1.20	0.13
Phenylalanine	1.73	1.64	0.81	0.25	1.24	2.22	1.77	1.58	0.49
Threonine	3.41	1.87	2.01	0.42	1.08	1.77	1.84	1.74	0.29
Valine	1.86	2.17	1.36	0.38	1.53	2.17	2.93	2.12	0.34
Total	**22.1**	**16.8**	**13.0**	**3.20**	**11.0**	**19.5**	**21.2**	**16.9**	**3.02**

Non-essential amino acids									
Aspartic acid	3.22	2.80	2.36	0.62	2.22	5.42	4.53	3.83	0.83
Alanine	2.39	0.95	1.55	0.20	1.36	1.91	4.92	3.78	0.26
Glutamic	8.54	7.92	5.26	2.90	3.95	9.67	6.65	4.99	2.70
Glycine	1.62	0.74	0.82	0.17	1.57	1.84	2.38	2.08	0.30
Proline	2.90	2.27	1.43	0.51	2.10	2.01	2.54	1.78	0.81
Serine	1.62	1.29	1.76	0.35	1.69	2.05	2.08	2.01	0.52
Tyrosine	1.48	1.13	1.23	0.25	0.99	1.60	2.65	2.86	0.25
Total	**21.8**	**17.1**	**14.4**	**5.0**	**13.9**	**24.5**	**25.7**	**21.3**	**5.67**

FM=fishmeal (FM), SBM=defatted soybean meal, BY=dry brewer's yeast, WF=wheat flour, CrM=cricket meal, BSFM=black soldier fly meal. Chemical composition of ingredients analysed by CelAgrid (Centre for Livestock and Agriculture Development), Cambodia.

[††]Chemical composition of ingredients analysed by Quality Testing Agriculture Forestry, Fisheries Centre, An Giang, Vietnam. Amino acids in Papers III and IV were analysed by National Institute of Animal Sciences, Hanoi, Vietnam.

Feed was offered by hand twice a day, at 3% and 5% of fish biomass in the hapa and tank experiment, respectively. The feeding rate was re-calculated between every sampling period based on exact weight increase between weighing periods. Fishmeal and de-fatted soybean meal were imported from Vietnam. The experimental diets were prepared by mixing the dry ingredients, then adding squid oil and finally incorporating sufficient distilled water to form a stiff dough that was run through a meat grinder to produce pellets. All diets were dried in the shade, then stored in a freezer until use. A new batch of diet was prepared every four weeks, with the same ingredients as the previous batch. The complete diets in the hapa and tank experiments were sent for analysis to the Centre for Livestock Agriculture and Development (CelAgrid), Cambodia, and the National Institute of Animal Sciences, Hanoi, Vietnam (Table 9).

In Paper IV, the formulation of the BSF meal diet was calculated to supply 45% crude protein and 15% lipid except in diet BSFM-3, which had 42% protein and 23% lipid due to the high lipid content in BSFM (Table 8). The experimental cricket and BSF meal diets were formulated with three levels of CrM (147, 300, and 443 g kg^{-1} %, denoted CrM-1, CrM-2 and CrM-3, respectively) and BSFM (222, 440 and 590 g kg^{-1}, denoted BSFM-1, BSFM-2 and BSFM-3, respectively), with fish meal as a control (Table 8). Feed was provided by hand until satiation, on two occasions per day (7:00 h and 16:00 h. Uneaten feed was collected an hour after feeding time and dried for calculation of feed eaten. Field crickets were bought from a small farm in Kandal province, Cambodia, while BSF pre-pupae were obtained from a local dealer in Vietnam. The experimental diet was prepared by mixing the dry ingredients together by hand, adding fish oil and then adding distilled water. The amount of water was adjusted to get the mixture to form a stiff dough, which was then pelleted at a size of 2.0 mm diameter. The pelleted diets were dried under the sun for 2-3 days until dry, then stored in a chest freezer until feeding. A new batch of diet was prepared every four weeks, using the same ingredients as in the previous batch. The final diets were sent for analysis of chemical composition (Table 9).

Table 8. *Diet formulation (g kg^{-1}) with inclusion of different levels of brewer's yeast (BY) (Paper III) and black soldier fly meal (BSFM) and cricket meal (CrM) (Paper IV)*

Parameter	Paper III†				Paper IV††						
	BY0	BY1	BY2	BY3	Control	BSFM-1	BSFM-2	BSFM-3	CrM-1	CrM-2	CrM-3
Fish meal	582	444	334	265	510	389	273	160	381	250	128
Soybean meal (defatted)	150	150	150	150	120	120	120	120	120	120	120
BY	0	204	366	468	-	-	-	-	-	-	-
CrM	-	-	-	-	-	-	-	-	147	300	443
BSFM	-	-	-	-	-	222	440	590	-	-	-
Wheat flour	163	97	45	12	230	190	142	80	238	239	239
Squid oil	85	85	85	85	-	-	-	-	-	-	-
Fish oil	-	-	-	-	50	50	50	50	50	50	50
Soybean oil	-	-	-	-	70	9	0	0	44	21	0
Carboxymethyl cellulose	10	10	10	10	10	10	10	10	10	10	10
Vitamin premix	10	10	10	10	10	10	10	10	10	10	10

†*Vitamin premix content per 500g: Vitamin A 350000 IU, vitamin D3 15000 IU, vitamin E 1350000 IU, vitamin B1 (thiamine) 500 mg, vitamin B2 (riboflavin) 500 mg, vitamin B6 (pyroxide) 500 mg, vitamin K 0.5 mg, biotin 15 mg, folic acid 150 mg, chlorine 50000 mg, d. capentothenate 1500 mg, copper 11000 mg, iron 22000 mg, zinc 11000 mg, cobalt 100 mg, calcium carbonate 150000 mg, manganese 3000 mg, nicotinamide 1000 mg, other 1 mg. Diet analysis by CelAgrid (Centre for Livestock and Agriculture Development), Cambodia.*

††*Soluble vitamin (name of product) manufactured by International nutrition, OMAHA, NE, USA): Net content 1kg: Vitamin A 6,000,000 IU, Vitamin D_3 500,000 IU, Vitamin E 4,000 IU, Menadione 1,000 mg, Vitamin B1 (Thiamine) 1,000 mg, Vitamin B2 (Riboflavin) 1,100 mg, Vitamin B6 (Pyridoxine) 500 mg, Vitamin B_{12} 5000 mg, Pantothenic acid 4,500 mg, Niacin 9,000 mg, Folic acid 400 mg, Biotin 30 mg, Vitamin C (Ascorbic acid) 3,000 mg. Diets analysis by National Institute of Animal Sciences, Hanoi, Vietnam.*

Table 9. *Chemical composition of the diet used in Paper III and Paper IV (% of dry matter, DM)*

Parameter	Paper III[†]								Paper IV[††]							
	Hapa				Tank											
	BY0	BY1	BY2	BY3	BY0	BY1	BY2	BY3	Control	SBM-1	SBM-2	SBM-3	CrM-1	CrM-2	CrM-3	Com[###]
DM	85.5	85.6	84.1	86.3	85.5	86.9	88.5	88.8	90.9	91.9	91.9	92.8	93.2	92.9	93.1	92.2
Crude protein	44.5	45.9	43.8	44.4	43.9	43.7	43.3	43.7	44.1	45.0	46.8	45.2	43.1	43.7	44.2	45.0
Crude fat	10.2	8.27	5.86	6.30	12.0	11.0	10.1	10.4	14.9	15.8	17.3	23.7	14.9	14.6	15.4	7.71
Crude fibre	1.06	1.15	1.07	1.06	2.8	5.3	4.1	3.3	3.63	5.07	6.97	7.35	3.59	4.45	5.06	2.35
Ash	13.6	11.2	10.2	8.92	15.1	12.8	11.2	10.1	10.8	10.5	9.59	9.04	8.92	7.66	6.21	10.6
ME[†], GE[†] (MJ/kg)	14.9	15.8	15.8	16.5	14.5	14.7	15.4	16.3	20.0	21.0	21.8	23.1	20.7	21.2	22.0	20.2
Essential amino acids (EAA)																
Arginine[#]	-	-	-	-	2.20	2.05	2.10	1.99	1.49	1.40	1.6	1.48	2.27	1.89	1.93	2.27
Histidine	-	-	-	-	0.75	0.79	0.70	0.65	0.18	0.32	0.48	0.25	0.54	0.48	0.40	0.85
Isoleucine	-	-	-	-	1.49	1.47	1.57	1.64	1.20	1.24	1.86	1.59	1.89	1.56	1.47	1.97
Leucine	-	-	-	-	3.00	2.98	2.91	2.95	1.93	1.99	2.18	2.12	2.81	2.44	2.47	2.77
Lysine[#]	-	-	-	-	3.50	3.52	3.54	3.50	1.08	1.34	1.76	1.90	2.10	1.78	1.89	2.84
Methionine[#]	-	-	-	-	1.05	0.95	0.81	0.74	0.47	0.73	0.71	0.88	0.90	0.64	0.81	1.03
Phenylalanine	-	-	-	-	1.98	1.95	1.96	1.80	1.30	1.22	1.44	1.29	1.74	1.38	1.31	1.61
Threonine	-	-	-	-	1.71	2.45	2.08	1.85	1.06	0.99	1.18	0.99	1.36	1.08	1.15	1.90
Valine	-	-	-	-	1.70	1.69	1.70	1.73	1.20	1.47	1.61	1.61	2.00	1.63	1.73	1.79
Total					**17.4**	**17.9**	**17.4**	**16.9**	**9.90**	**10.7**	**12.8**	**12.1**	**15.6**	**12.9**	**13.2**	**17.0**

Non-essential amino acids																
Aspartic	-	-	-	-	3.69	3.58	3.72	3.78	2.22	2.48	2.82	2.51	3.07	2.78	2.90	3.88
Alanine	-	-	-	-	2.53	2.53	2.42	2.41	1.11	1.33	1.64	1.64	2.01	2.01	2.46	1.92
Glutamic	-	-	-	-	6.36	6.52	6.45	6.26	5.01	4.55	4.74	3.98	6.29	5.36	5.34	7.55
Glycine	-	-	-	-	2.17	1.65	1.77	1.76	1.30	1.35	1.5	1.47	1.79	1.42	1.51	2.03
Proline	-	-	-	-	2.36	2.00	2.50	2.63	1.66	1.66	1.77	1.73	2.37	1.93	1.74	1.84
Serine	-	-	-	-	1.57	1.66	1.78	1.84	1.81	1.23	1.27	0.78	1.78	1.38	1.50	1.63
Tyrosine	-	-	-	-	1.34	1.39	1.28	1.28	0.96	1.27	1.7	1.75	1.59	1.36	1.47	1.23
Total					**20.0**	**19.3**	**19.9**	**20.0**	**14.1**	**13.9**	**15.4**	**13.9**	**18.9**	**16.2**	**16.9**	**20.1**

BY0, BY1, BY2 and BY3 = Brewer's yeast at 0 (Control), 0, 20, 37 and 47 % fishmeal replacement; Black soldier fly meal at level 1, 2 and 3 levels 222, 440 and 590 g kg^{-1} of fishmeal replacement denotes as BSFM-1, BSFM-2 and BSFM-3; Cricket meal at level 147, 300 and 443g kg^{-1} of fishmeal replacement denotes as CrM-1, CrM-2 and CrM-3 Com.$^{\#\#}$ = Commercial dried pellet chemical composition: CP 42%, moisture 11%, lipid 5-7%, fibre 4%, Ca 1-3%, P 1-2%, lysine 2.14%, methionine 1.2%, energy 2500 kcal kg^{-1} as indicated by manufacture, CP Group, Vietnam Co.; †Chemical composition (% DM) and metabolisable energy (ME) content (MJ kg-1) of the diets in hapa and tank performed by CelAgrid (Centre for Livestock and Agriculture Development), amino acid analyses (tank experiment) performed by National Institute of Animal Sciences, Hanoi, Vietnam.; ††Chemical composition (% DM) and gross energy (GE) content (MJ kg-1) analysed by Quality Testing Agriculture Forestry, Fisheries Centre, An Giang, Vietnam.; EAA$^{\#}$ Based on requirement for Asian seabass (% of protein) according to Millamena (1996): arginine 3.8%, lysine 4.5%, methionine 2.24%, and Murillo-Gurrea et al (2001): lysine and arginine were estimated to be 20.6 g kg^{-1} diet (4.5% of protein) and 18.2 g kg^{l}diet (3.8% of protein), respectively.

3.2.3 Data collection

In all experiments in Papers II and III, the fish were weighed before, during and after the experiments. Total amounts of feed eaten and uneaten were recorded and the feed conversion ratio (FCR) was calculated. Weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), survival rate (SR) and condition factor were calculated. Water parameters such as temperature, dissolved oxygen (DO) and pH were measured in each hapa or tank twice a day. In Paper II, salinity was measured and adjusted where necessary.

In Paper IV, in addition to the parameters listed above, viscero-somatic index (VSI) and hepato-somatic index (HIS) were determined. At the start, five fish (representative sample for initial body composition) were sampled and frozen as a starting reference for body proximate chemical analyses. At week 6 and the end of week 9, three fish per replicate (9 fish per treatment) were randomly anaesthetised with an overdose of ethylene glycol mono-phenyl ether at a concentration of 0.5 ml L^{-1} and then frozen and stored for analysis of body proximate chemical analysis.

3.2.4 Chemical analysis

In all trials, feed samples were analysed in duplicate or triplicate. In Papers III and IV, feed samples were analysed in duplicate as follows: dry matter was determined by drying in an oven at 105 °C until constant weight. Ash content was determined by incineration of the sample at 550 °C for 4 h. Crude protein was calculated as 6.25 × % N analysed by the Kjeldahl method, ether extract (EE) was measured using the Soxhlet method and crude fibre (CF) content was analysed using standard methods (AOAC, 2000). Amino acid content was determined by high performance liquid chromatography (HPLC) according to Vázquez-Ortiz *et al.* (1995). Nitrogen-free extract (NFE) was calculated according to methods described in AOAC (2000) (NFE = 100 – (% protein + % lipid + % fibre + % ash)). Gross energy content (MJ kg^{-1}) was calculated according to NRC (1993), using a value of 5.64, 9.44 and 4.11 kcal g^{-1} whole body for protein, lipid and carbohydrate, respectively.

3.2.5 Statistical analysis

Survey data in Paper I were coded and analysed by descriptive statistics and analysis of variance, using Microsoft EXCEL and SAS (Version 9.4). Differences were considered significant at $p<0.05$.

In Paper II, the data were analysed using general linear model analysis of variance (GLM ANOVA) in Minitab version 17.

In Paper III and IV, all growth performance data were statistically analysed by using General Linear Model (GLM) one-way (ANOVA) using SAS (Statistical analysing system, ver. 9.4). Turkey pairwise comparison was used

as *post-hoc* test where the complete model was found to be significant and $p < 0.05$ was considered significant. The fixed factor was the treatments and, where appropriate, body weight was used as co-variate in paper IV. All data were tested a priori for normal distribution. Data points outside the 97.5% confidential interval were omitted from the statistical analysis (three data points in all, deviating by 10 or 100 times from the mean indicating clerical errors).

3.3 Digestibility experiment

3.3.1 Experimental conditions

A digestibility experiment on all feeds used in Papers III (spent brewer yeast) and paper IV (black soldier fly and cricket) were carried out in August 2018 at An Giang University, in a closed recirculation system (RAS system). Twelve parallel-connected composite settlement tanks, with a volume of about 500 L per tank, were connected to a sedimentation tank containing sand (80 cm layer) that functioned as a mechanical filter. Water was circulated in the rearing tanks; new water comprising around 30% with 10 psu (practical salinity unit) was added to the sedimentation tank every day. A total of 396 fingerlings at an average size of 33 g, from a site in Nha Trang province located around 600 km from An Giang University, were distributed between the tanks (stocking density 33 fingerlings per tank) and fed the test diet for 10 days before the start of faeces collection. Aeration with one air-stone was provided to individual tanks via low-pressure electrical blowers. The sedimentation tubes were connected by a funnel at the bottom of each tank, where the fish faeces settled, and the base of each tank was surrounded with ice and salt to keep the temperature at 4-5 °C and thereby minimise microbial degradation of the faeces. In addition, the experimental system tanks and biological filter tank were covered with white plastic (polyethylene) sheeting to prevent dust and insects from entering the fish tanks. The walls of tanks in the culture system and in the biological filter system were cleaned to remove slime from fish and microbes twice a week. Dissolved oxygen, temperature and ammonia (NH_3) were measured every two days in each tank (at 7:00-8:00 h and 13:00-14:00 h), using a dissolved oxygen meter (model 407510, EXTECH), a pH/temperature meter (EXTECH) and NH_3 and NO_2 kits (Sera test kits). Salinity was measured daily using a digital hydrometer and adjusted when necessary.

3.3.2 Experimental diets

Four dietary treatments were evaluated, one reference diet and three experimental diets (Table 10). Each diet was tested in triplicate units. Fish were fed two meals daily (at 7:00 h and 16:00 h) to visual satiety (until the first feed

item was refused) and allowed to adapt to the diet over the 10-day period prior to faeces collection. The three test diets were prepared containing 70% reference diet and 30% test ingredient (spent brewer's yeast, cricket meal and BSF meal), based on fish requirements stated in Cho *et al*. (1982). The reference diet was formulated to contain 45% crude protein and 16% lipid. Indigestible fibre was used as an internal reference marker (Tacon *et al*., 1984). The chemical composition and amino acid profile of the experimental diets are given in Table 10 and that of the ingredients can be found in Table 7.

Table 10. *Formulation (g kg^{-1}) of the diets in the digestibility experiments*

Ingredient (%)	Control	CrM	BSFM	BY
Fish meal	510	357	357	357
Soybean meal	120	84	84	84
Cricket meal, CrM	-	300	-	-
Black soldier fly meal, BSFM	-	-	300	-
Brewer's yeast, BY	-	-	-	300
Wheat flour	230	161	161	161
Fish oil	50	35	35	35
Soybean oil	70	49	49	49
Carboxymethyl cellulose	10	7	7	7
Vitamins[†]	10	7	7	7
Chemical composition of the diets (% DM) and energy content (MJ kg^{-1}). (Analyses performed by National Institute of Animal Sciences, Hanoi, Vietnam)				
Moisture	14.0	13.9	15.9	14.1
Crude protein	43.2	48.0	44.5	43.4
Lipid	15.8	18.0	22.1	10.6
Ash	12.8	11.2	11.3	10.7
Fibre	3.09	4.69	5.83	3.66
Energy	20.9	21.7	22.1	20.0
Essential amino acids				
Arginine	1.51	2.23	1.63	1.72
Histidine	0.44	0.59	0.58	0.49
Isoleucine	1.05	1.30	1.19	1.25
Leucine	1.92	2.46	2.21	2.07
Lysine	0.95	1.44	1.25	1.26
Methionine	0.37	0.57	0.59	0.38
Phenylalanine	1.22	1.42	1.31	1.35
Threonine	0.91	1.16	1.14	1.27
Valine	1.07	1.54	1.39	1.29
Total	**9.44**	**12.7**	**11.3**	**11.1**

Non-essential amino acids				
Aspartic	1.80	2.39	2.12	2.24
Alanine	1.16	2.26	1.80	1.46
Glutamic	4.42	5.28	4.43	4.92
Glycine	0.94	1.25	1.18	1.09
Proline	1.33	1.51	1.32	1.34
Serine	2.14	1.71	1.40	1.51
Tyrosine	1.08	1.39	1.50	1.02
Total	**22.3**	**28.5**	**25.0**	**24.7**

Soluble vitamin (name of product) manufactured by International nutrition, OMAHA, NE, USA), Net content 1kg: Vitamin A 6,000,000 IU, Vitamin D_3 500,000 IU, Vitamin E 4,000 IU, Menadione 1,000 mg, Vitamin B1 (Thiamine) 1,000 mg, Vitamin B2 (Riboflavin) 1,100 mg, Vitamin B6 (Pyridoxine) 500 mg, Vitamin B_{12} 5000 mg, Pantothenic acid 4,500 mg, Niacin 9,000 mg, Folic acid 400 mg, Biotin 30 mg, Vitamin C (Ascorbic acid) 3,000 mg.

3.3.3 Faeces collection

Fish faeces samples were collected twice a day for 20 days from the faecal settling tube before new feeding (*i.e.* at 7:00 h and 16:00 h). Every day, uneaten feed was flushed out by opening the valve of the faecal collection tube before feeding and then new water was added, so that at least 40% of the water in the experimental tanks was changed daily. The faeces samples were slowly flushed out by opening the valve of the faeces collection tube and filtering through a 30 µm mesh cloth. Faeces samples from each tank were pooled in sealed pots and kept frozen until the end of the collection period pending chemical analysis.

3.3.4 Digestibility analysis

Apparent digestibility (AD) of dry matter, protein, lipid, gross energy and amino acid for the reference and test diets was calculated as described by Cho (1979):

ADC_{diet} (%) = [1 - (% $CF_{dietary}$ / % CF_{faeces}) x (% $Nutrient_{faeces}$ / % $Nutrient_{diet}$)] x 100

Where: $CF_{dietary}$ and CF_{faeces} are the crude fibre content of the diet and faeces, respectively, and

$Nutrient_{diet}$ and $Nutrient_{faeces}$ are the nutritional parameters (dry matter, protein, amino acid or energy) of the diets and faeces, respectively.

Crude fibre was used as the inert marker suggested by Tacon and Rodrigues (1984).

3.3.5 Chemical analysis

Samples of feed ingredients, diets and faeces were analysed in duplicate using standard methods (AOAC, 2000) for dry matter, crude fibre, ash, total lipid, nitrogen-free extract, amino acid and gross energy content. Dry matter was determined by drying in an oven at 105 °C for 24 h. Nitrogen content was

determined by the Kjeldahl method and crude protein was calculated as N x 6.25. Crude fat (EE) content was analysed using the Soxhlet method after acid hydrolysis of the sample. Ash content was determined by incineration in a muffle furnace at 550 °C for 12 h.

All digestibility data were statistically analysed by GLM one-way ANOVA by Turkey pairwise comparison, using Minitab software version 17 ($p<0.05$ considered significant), with treatment as the fixed factor.

Main results

4.1 Survey (Paper I)

Trash fish are commonly used as a feed for Asian seabass and the survey showed that a majority of fish farmers in Cambodia still rely on this feed resource. The survey also showed that the floating net cage was most common farming method in Preah Sihanoukvill, while the stationary cage was common in Kampot and Koh Kong provinces (Table 11) (Figure 2 and 3). A higher percentage of farmers surveyed in Preah Sihanoukvill owned net cages, and pond and cage culture were most common in that province. However, the ponds were reported to be used only for small fingerlings (Table 11).

Farmers said that they face some problems with fish mortality during the culture period. Parasites (white and dark leeches) were reported to cause mortality in all provinces. The incidence of scale drop syndrome was reported to be around 55% and 60% in Preah Sihanoukvill and Koh Kong province, respectively (Table 12). It was reported that fish mortality occurs in the fish at weight around 20-50 g and peaks again when the fish reach a weight of 200-350 g, in both the rainy and dry season.

Table 11. *Characteristics of cages and ponds used in small-scale Asian seabass farming in the three Cambodian provinces surveyed in Paper I. Values shown are mean (min – max)*

Characteristic	Province			p-value	Standard error
	Preah Sihanoukvill[a]	Kampot[b]	Koh Kong[c]		
Cages number	60 (8.0-300)	7.0 (1.0-23)	6.0 (1.0-20)	0.0002	42.8
Cage size (m^2)	8.0 (3.0-16)	13.7 (4.0-36.8)	14.4 (6.3-27)	0.0005	5.3
Cage depth (m)	2.4 (2.0-3.0)	2.8 (2.0-3.5)	3.5 (2.0-5.5)	<0.0001	0.7
Pond number	5.0 (3.0-6.0)	2.0 (2.0-2.0)	2.0 (1.0-3.0)	0.1	1.2
Pond size (m^2)	5761.0 (576-5000)	750.0 (600-900)	7200.0 (1600-10000)	0.3	3961.7
Pond depth (m)	1.0 (1.0-1.5)	1.5 (1.5-1.5)	1.3 (1.0-1.5)	0.3	0.2
Characteristics of stocking in small-scale Asian seabass farms in the three provinces					
Cage, smaller size (head/cage)	880 (100-4000)	1468 (250-800)	1782 (380-5000)	0.2	1641.5
Cage, larger size (head/cage)	328 (100-600)	448 (250-550)	460 (250-2000)	0.002	122.1
Pond, smaller size (head/pond)	964 (600-10000)	2859 (10000-10000)	1156 (5000-10000)	0.49	3487.4
General information on small-scale Asian seabass farming					
Experience (year)	9.7 (1-20)	5.6 (2-16)	6.6 (1-32)	0.2	6.4
Size of fingerlings (cm)	7.0 (4-12)	8.0 (6-12)	6.2 (4-7.5)	0.02	1.7
Price of fingerlings ($/head)	0.3 (0.15-0.40)	0.3 (0.15-0.33)	0.2 (0.13-0.23)	<0.0001	0.06
Culture period (month)	9 (4-12)	7 (6-10)	8 (5-12)	0.04	1.9
Harvest size (g)	877 (600-1100)	977 (750-1200)	854 (650-1100)	0.05	137.3
Selling price of fish ($/kg)	6.4 (4.0-8.0)	5.7 (5.0-7.0)	4.4 (3.2-7.0)	<0.0001	0.8
Survival rate (%)	30.8 (5-50)	49.5 (25-60)	20.2 (5-60)	<0.0001	15.0
Cost of feed (trash fish) ($/kg)	0.3 (0.18-0.31)	0.3 (0.18-0.33)	0.2 (0.16-0.33)	0.02	0.1
Fingerling per crop (head)	400 (300-60000)	575 (400-15000)	503 (380-30000)	0.1	7912.0

Expenditure and income				
Total cost of fingerling ($/ crop)	3049 (75-13650)	996 (130-4875)	1468 (74.10-5850)	0.02	2172.6
Total cost of trash fish ($/day)	41.9 (1.2-300)	8.9 (1.75-48.75)	9.8 (0.81-58.5)	0.04	42.8
Total cost of labour ($/month)	200 (70-700)	150 (100-200)	200 (200-200)	0.09	151.6
Total harvest (kg)	969 (40-5000)	251.8 (33-1000)	482.5 (75-4500)	0.2	967.5
Total income from selling($/cycle)	5127.9 (240-29250)	1509.0 (165-6000)	2181.4 (244-20475)	0.16	5849.8

Number of farmers ([a]n= 20), ([b]n=11), ([c]n=25).

Table 12. *Chemical composition of the diets fed at different salinity levels (psu) in Paper II. Values shown are mean ± standard deviation (SD)*

Parameter		Fry						Fingerling				
		0psu	5psu	10psu	20psu	p-value	SD		10psu	20psu	30psu	p-value
No. of fish (head)	Initial	100	100	100	100	-	-		10	10	10	-
	Final	97	100	99	100	0.27	1.46		10	10	10	-
Body weight (g)	Initial	0.32	0.32	0.32	0.33	> 0.1	0.01		7.7±1.82	6.0±0.22	7.9±1.37	0.25
	Final	1.88	1.99	1.86	1.98	> 0.1	0.07		60.4±14.0	49.8±13.3	52.3±16.4	0.67
Daily weight gain (g d^{-1})*		0.11	0.12	0.11	0.12	0.25	0.004		-	-	-	-
Feed conversion ratio		0.63	0.60	0.64	0.63	> 0.1	0.01	Initial	0.53±0.03	0.46±0.05	0.55±0.05	0.10
		-	-	-	-	-	-	Final	1.70±0.61	2.74±2.38	2.27±1.61	0.77
Specific growth rate (% d^{-1})		-	-	-	-	-	-		3.67±1.33	3.73±1.74	3.34±0.91	0.75
Condition factor		-	-	-	-	-	-	Initial	1.52±0.13	1.55±0.03	1.40±0.01	0.11
		-	-	-	-	-	-	Final	1.41±0.14	1.53±0.09	1.42±0.17	0.52

Note: Daily weight gain = (Final weight, g – Initial weight, g) / Number of days; Feed conversion ratio = (Total feed, g / Weight gain, g); Specific growth rate = (Ln $_{Final\ body\ weight}$ *– Ln* $_{Initial\ body\ weight}$*) / Number of days x 100; Condition factor (Body weight, g / Body length*^{3}*, cm) x 100.*

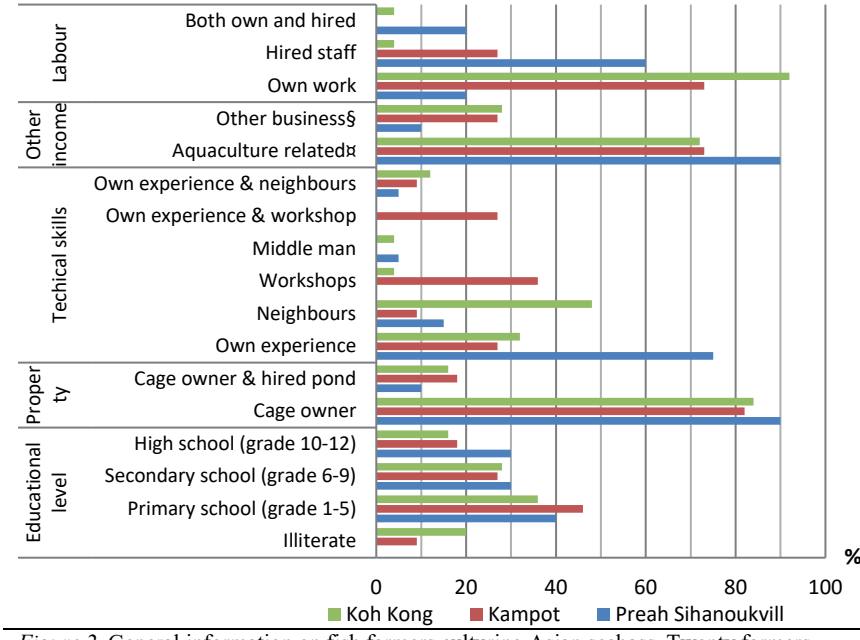

Figure 2. General information on fish farmers culturing Asian seabass. Twenty farmers from three provinces, Koh Kong, Kampot and Preah Sihanoukvill, located on the coast of Cambodia, were interviewed to gather information about labour, income and skills.

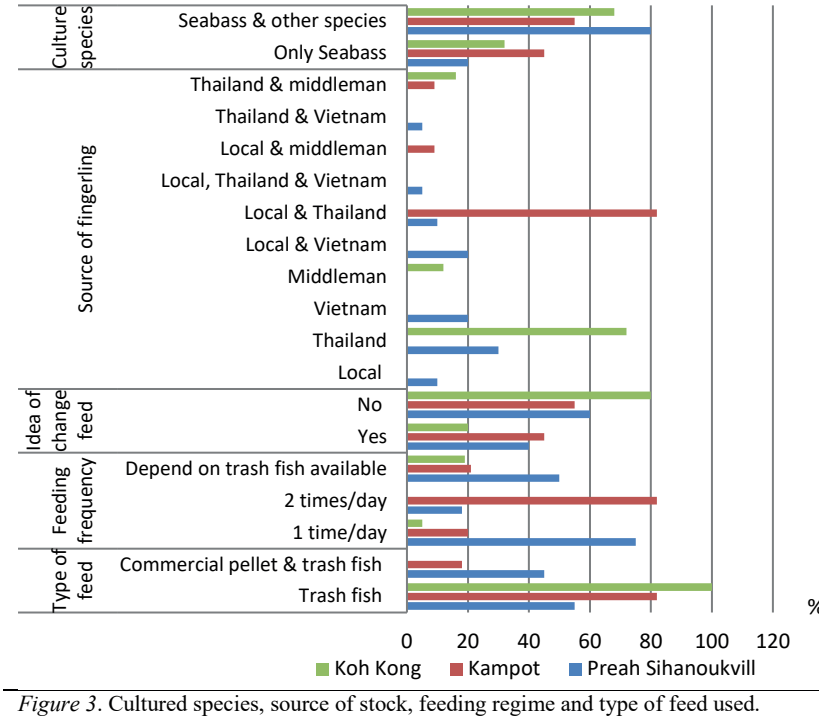

Figure 3. Cultured species, source of stock, feeding regime and type of feed used.

4.2 Growth performance (Papers II-IV)

The fish in Paper II showed much better weight gain than in other experiments, increasing by almost 5- to 6-fold their initial weight within 56 days. No mortality occurred in fingerlings, but there were some losses in fry, caused by cannibalism. There were no differences in growth, FCR and condition factor between the treatments in Paper II (Table 12). The FCR was <1 in fry and >2 in fingerlings (final period), but the difference between treatments was not significant ($p>0.1$ and $p=0.77$ in fry and fingerlings, respectively.

In Paper III, the trials in hapas and tanks were conducted at different times and with different sizes of fingerlings (>40 g in hapas, >20 g in tanks). Therefore, no comparison was made of the hapa and tank systems. There were no significant differences at any level of brewer's yeast used to replace the fishmeal in the diet (Table 13). The fish doubled or tripled in weight during the trial period of 2.5 months in both hapas and tanks, with no difference between the treatments. However, DWG (g day^{-1}) in both the hapa and tank trials seemed to decrease in the final period, with no significant difference between the treatments ($p=0.9$ for hapas, $p=0.36$ for tanks). The survival rate in both experiments was 90-97% in hapa, 93-97% in tank experiment and was not significantly different between diets (Table 13). A non-statistically significant difference in total feed given and FCR between low and high yeast inclusion was observed in the hapas experiment (Table 13). Furthermore, FCR was generally higher in hapas than in tanks. However, the FCR calculation for hapas was based on total feed provided, since the uneaten feed could not be collected. In contrast, care was taken to overfeed the fish in the tank experiment. On the other hand, starting weight of the fish was higher in the hapas experiment.

In Paper IV, there were no differences between the treatments at any level of inclusion of either insect meal tested (BSFM and CrM). On average, the fish doubled in body weight during the experiment, with a rather high FCR (Table 14). There were no consistent differences in growth, survival or FCR between the diets. Moreover, HSI and VSI did not differ between treatments when body weight (BW) was included as a co-variate in the statistical model. The only dietary-related difference ($p<0.05$) in body composition was found for crude protein (CP) and lysine content. Fish fed the control and CrM-2 diet displayed a lower CP content compared with that at the start of the experiment. This was significant when compared with the BSFM-2 diet, where the fish displayed the highest value of body CP content at the end of the experiment (Table 14).

Table 13. *Chemical composition of the diets containing different levels of brewer's yeast (BY) fed to fish in hapas and tanks in Paper III. Values shown are mean ± standard error (SE)*

Parameter	Period	Hapa					Tank				
		BY0	BY1	BY2	BY3	p-value	BY0	BY1	BY2	BY3	p-value
No. of fish (head)	Initial	70	70	70	70	—	45	45	45	45	—
	Final	66 ± 1.34	63 ± 1.34	68 ± 1.34	68 ± 1.34	0.07	42 ± 1.14	43 ± 1.14	43 ± 1.14	44 ± 1.14	0.67
Body weight (g)	Initial	41.2 ± 3.65	41.0 ± 3.65	44.8 ± 3.65	44.6 ± 3.65	0.81	22.9 ± 0.47	23.3 ± 0.47	22.7 ± 0.47	22.4 ± 0.47	0.56
	Final	114.8 ± 9.79	109.5 ± 9.79	109.7 ± 9.79	103.8 ± 9.79	0.89	99.7 ± 2.96	97.7 ± 2.96	94.0 ± 2.96	91.2 ± 2.96	0.26
Daily weight gain (g d^{-1})	Initial	1.16 ± 0.13	1.01 ± 0.13	1.04 ± 0.13	0.97 ± 0.13	0.73	1.07 ± 0.06	0.97 ± 0.06	0.93 ± 0.06	1.00 ± 0.06	0.54
	Final	0.64 ± 0.11	0.54 ± 0.11	0.60 ± 0.11	0.63 ± 0.11	0.91	0.77 ± 0.12	1.07 ± 0.12	0.93 ± 0.12	0.77 ± 0.12	0.30
	Final	1.23 ± 0.11	1.14 ± 0.11	1.08 ± 0.11	0.99 ± 0.11	0.50	1.28 ± 0.10	1.23 ± 0.10	1.19 ± 0.10	1.16 ± 0.10	0.84
Feed conversion ratio		1.5 ± 0.20	1.7 ± 0.20	1.8 ± 0.20	1.8 ± 0.20	0.54	1.2 ± 0.08	1.3 ± 0.08	1.3 ± 0.08	1.4 ± 0.08	0.33
Survival rate (%)	Final	94.3 ± 1.87	90.0 ± 1.87	97.3 ± 1.87	97.3 ± 1.87	0.06	93.0 ± 2.57	95.7 ± 2.57	95.7 ± 2.57	97.3 ± 2.57	0.70
Total feed (g)		1652 ± 107	1616 ± 107	1782 ± 107	1772 ± 107	0.61	984 ± 81.4	1042 ± 81.4	1013 ± 81.4	1052 ± 81.4	0.93
Condition factor	Initial	-	-	-	-	-	1.20 ± 0.03	1.27 ± 0.03	1.23 ± 0.03	1.23 ± 0.03	0.49
	Final	-	-	-	-	-	1.5	1.5	1.5	1.5	—

Daily weight gain = (Final weight (g) – Initial weight (g))/Number of days; Feed conversion ratio = (Total feed, g/Weight gain, g);

Survival rate = (Final number of fish / Initial number of fish) x 100; Specific growth rate = (Ln $_{Final\ body\ weight}$ – Ln $_{Initial\ body\ weight}$) / Number of days x 100;

Condition factor = (Body weight (g) / Body length^3 (cm)) x100

BY0, BY1, BY2 and BY3 = brewer's yeast at 0 (fishmeal as control), 20, 37 and 47 % DM replacement of fishmeal, respectively,

Table 14. *Chemical composition (mean ± standard deviation (SD)) of the diets containing different levels of black soldier fly meal (BSFM) and cricket meal (CrM) fed to fish in Paper IV, and of the fish body (analyses performed by National Institute of Animal Sciences, Hanoi, Vietnam)*

Parameter		Control	BSFM-1	BSFM-2	BSFM-3	CrM-1	CrM-2	CrM-3	p-value
Body weight (g)	Initial	12.2 ± 0.82	11.4 ± 1.95	11.6 ± 2.4	11.7 ± 1.35	11.7 ± 0.85	11.8 ± 1.91	11.6 ± 1.87	0.99
	Final	21.5 ± 2.94	26.2 ± 2.31	18.9 ± 3.8	18.4 ± 1.81	21.3 ± 0.23	22.3 ± 5.71	22.7 ± 5.75	0.22
Feed conversion ratio		3.86 ± 0.94^{a}	2.45 ± 0.6^{c}	2.51 ± 0.64^{bc}	2.31 ± 0.41 ^{c}	3.08 ± 1.03^{abc}	3.41 ± 1.69^{ab}	2.40 ± 0.99^{c}	0.001
Specific growth rate (% d^{-1})		0.89 ± 0.52	1.33 ± 0.50	0.78 ± 0.29	0.72 ± 0.22	0.96 ± 0.75	0.99 ± 0.29	1.04 ± 0.54	0.16
Viscero-somatic index (%)		6.48 ± 1.79	6.40 ± 1.14	5.40 ± 0.38	5.91 ± 1.46	5.63 ± 0.76	6.23 ± 0.55	5.85 ± 0.98	0.88
Hepato-somatic index (%)		1.21 ± 0.56	1.52 ± 0.34	1.36 ± 0.35	1.61 ± 0.61	1.39 ± 0.80	1.18 ± 0.26	1.12 ± 0.54	0.88
Survival rate (%)		92.5 ± 12.8	97.1 ± 5.42	93.8 ± 10.3	95.8 ± 7.64	87.1 ± 18.4	92.9 ± 10.8	90.4 ± 15.6	0.50

Chemical composition (%DM) of fish body, analysed by LSMean, corrected for body weight. Values shown are mean ± standard error (SE)

	Initial	Final							
Moisture	71.2	72.6 ± 0.49	72.0 ± 0.71	73.0 ± 0.70	72.6 ± 0.70	72.6 ± 0.69	71.6 ± 0.69	72.8 ± 0.69	0.79
Crude protein	62.9	60.8 ± 0.57^{b}	62.6 ± 0.82^{ab}	63.7 ± 0.81^{a}	62.8 ± 0.81^{ab}	61.6 ± 0.81^{ab}	60.8 ± 0.81^{b}	62.2 ± 0.81^{ab}	0.05
Lipid	21.5	20.0 ± 0.92	20.4 ± 1.32	18.9 ± 1.31	20.4 ± 1.31	20.0 ± 1.30	22.2 ± 1.30	18.1 ± 1.30	0.43
Ash	14.6	18.5 ± 0.71	16.3 ± 1.03	17.8 ± 1.01	16.8 ± 1.02	18.4 ± 1.01	16.7 ± 1.01	19.4 ± 1.01	0.25
Energy, MJ/kg	23.4	22.3 ± 0.29	22.9 ± 0.42	22.5 ± 0.42	22.9 ± 0.42	22.5 ± 0.41	23.2 ± 0.41	21.9 ± 0.41	0.32

Essential amino acids									
Arginine	4.92	4.45 ± 0.14	4.59 ± 0.20	4.45 ± 0.20	4.51± 0.20	4.48 ± 0.20	4.65 ± 0.20	4.61 ± 0.20	0.98
Histidine	1.73	1.56 ± 0.07	1.73 ± 0.10	1.81 ± 0.10	1.52 ± 0.10	1.72 ± 0.10	1.62 ± 0.01	1.79 ± 0.10	0.23
Isoleucine	2.32	2.00 ± 0.05	2.17 ± 0.08	2.09 ± 0.08	2.18 ± 0.08	1.89 ± 0.08	2.03 ± 0.08	2.05 ± 0.08	0.13
Leucine	4.1	3.59 ± 0.07	3.84 ± 0.10	3.69 ± 0.10	3.79 ± 0.10	3.50 ± 0.10	3.68 ± 0.10	3.77 ± 0.10	0.14
Lysine	4.65	4.41 ± 0.12^{b}	4.67 ± 0.17^{ab}	4.86 ± 0.17^{ab}	5.03 ± 0.17^{a}	4.43 ± 0.17^{b}	4.66 ± 0.17^{ab}	4.85 ± 0.17^{ab}	0.04
Methionine	1.55	1.24 ± 0.08	1.35 ± 0.11	1.22 ± 0.11	1.15 ± 0.11	1.23 ± 0.11	1.42 ± 0.11	1.18 ± 0.11	0.64
Phenylalanine	2.35	2.08 ± 0.06	2.22 ± 0.09	2.04 ± 0.09	2.27 ± 0.09	2.14 ± 0.09	2.21 ± 0.09	2.20 ± 0.09	0.40
Threonine	2.9	2.93 ± 0.10	2.87 ± 0.14	2.90 ± 0.14	2.97 ± 0.14	2.99 ± 0.14	2.94 ± 0.14	2.98 ± 0.14	1.00
Valine	2.48	2.23 ± 0.05	2.35 ± 0.07	2.28 ± 0.07	2.39 ± 0.07	2.14 ± 0.07	2.25 ± 0.07	2.31 ± 0.07	0.26
Non-essential amino acids									
Aspartic	5.13	5.17 ± 0.12	5.27 ± 0.18	5.32 ± 0.17	5.48 ± 0.17	5.10 ± 0.17	5.25 ± 0.17	5.34 ± 0.17	0.76
Alanine	4.16	3.94 ± 0.06	4.10 ± 0.09	4.03 ± 0.09	4.03 ± 0.09	3.90 ± 0.09	4.01 ± 0.09	4.18 ± 0.09	0.30
Glutamic	10.2	9.13 ± 0.17	9.61 ± 0.24	9.53 ± 0.24^{a}	9.60 ± 0.24	8.92 ± 0.24	9.30 ± 0.24	9.59 ±0.24	0.19
Glycine	3.91	4.10 ± 0.14	4.18 ± 0.19	4.37 ± 0.19	4.42 ± 0.19	4.10 ± 0.19	3.91 ± 0.19	4.61 ± 0.19	0.16
Proline	2.39	2.25 ± 0.07	2.27 ± 0.11	2.31 ± 0.10	2.22 ± 0.11	2.17 ± 0.10	2.34 ± 0.10	2.37 ± 0.10	0.83
Serine	2.5	2.31 ± 0.08	2.48 ± 0.12	2.34 ± 0.12	2.38 ± 0.12	2.30 ± 0.12	2.33 ± 0.12	2.45 ± 0.12	0.87
Tyrosine	1.72	1.56 ± 0.03	1.65 ± 0.05	1.65 ± 0.05	1.67 ± 0.05	1.52 ± 0.05	1.61 ± 0.05	1.63 ± 0.05	0.23

*Specific growth rate = (Ln Measured weight - Ln Previous measured weight) / No. of days between samplings) x 100;); Survival rate = (Final number of fish / Initial number of fish) x 100; Specific growth rate = (Ln $_{\text{Final body weight}}$ − Ln $_{\text{Initial body weight}}$) / Number of days x 100; conversion ratio = Total feed intake, g/Total wet weight gain, g; Hepatosomatic index = 100 x (Liver weight / Body weight); Viscero-somatic index = 100 x (Viscera weight / Body weight). BSFM-1, BSFM-2, BSFM-3 = Black soldier fly meal at 222, 440 and 590 g kg^{-1} replacement of fishmeal, respectively; CrM-1, CrM-2 and CrM3 = Cricket Meal at 147, 300 and 443 g kg^{-1} replacement of fishmeal, respectively. *Different superscripts within rows indicate significant ($p<0.05$) difference between treatments.*

4.3 Digestibility study (this thesis)

The digestibility results revealed that there were significant differences in crude protein digestibility and energy between the different diets (Table 15).

Table 15. *Apparent digestibility (% DM) of the test diets, determined using crude fibre as internal marker. Values shown are mean ± standard deviation (SD)*

Diet	AD of dietary		
	Crude protein	Lipid	Energy
Black soldier fly meal	55.2 ± 3.3[b]	84.8 ± 1.7	71.0 ± 1.7[b]
Cricket meal	63.4 ± 7.2[b]	79.3 ± 5.1	71.4 ± 5.5[b]
Brewer's yeast	81.5 ± 1.7[a]	81.7 ± 3.2	83.1 ± 1.9[a]
Control	82.2 ± 1.1[a]	86.3 ± 0.1	84.0 ± 0.8[a]
p-value	< 0.001	0.1	0.001

5 General discussion

5.1 Main research topics in the thesis

This thesis examined the status of marine aquaculture in Cambodia in general and the problem of non-sustainable use of trash fish as feed for Asian seabass in particular. Cambodia has one of the highest rates of fish consumption per capita globally, with estimated values of up to 80 kg per year. Fish thereby constitute a major protein resource with special importance for the resource-poor sector of the population. At present, major development for hydropower is ongoing in the Mekong river, threatening the spawning migration of the majority of the main wild food fish species in Cambodia. Development of sustainable aquaculture is therefore of national interest in Cambodia. However, the farming industry is in major need of improved and environmentally friendly farming practices. The industry is also currently dependent on imported fabricated feeds, based on soy and fish ingredients. Such diets are expensive and can constitute up to 90% of the total production costs for small-scale farmers, and are also difficult to obtain as smallholder farmers have limited market access. This has prompted many such farmers to use locally obtained trash fish, often harvested from threatened populations. Furthermore, the low hygiene and risk of oxidation of these products introduce risks of toxins and pathogens to the fish.

The survey in Paper I determined the current state of Cambodian Asian seabass farming, including use of fabricated diets, trash fish, location and production volume of systems in the different coastal regions of Cambodia.

The study in Paper II examined whether differing saline environments in coastal farming are a major factor in the production of Asian seabass juveniles. This information is vital in formulation of a national strategy, as fish farming is currently situated in marine and brackish waters and freshwater bodies. Asian seabass is an euryhaline species, tolerating a wide variety of salinities.

The study showed a slight (non-significant) preference for isotone salinity, but all salinities tested supported juvenile growth and survival.

In Paper III, the possibility of using spent brewer's yeast, a local industrial by-product and well-controlled source of low-cost protein, as a feed ingredient for Asian seabass was investigated. The study was conducted both in hapas (small cages inside a larger tank) and in tanks. Irrespective of rearing environment, brewer's yeast was found to be suitable as a partial replacement for protein from fish and soy in feeds for Asian seabass juveniles.

In Paper IV, two types of insects, local crickets and black soldier fly larvae (BSF), were tested as complementary and low-resource sources of protein and lipids in the diet of Asian seabass. Both these insects were shown to function well on different waste streams available in Cambodia, *e.g*. cassava tops, food/fish waste *etc*. Both insects have potential to be produced in large- or small-scale production units. In general, both sources functioned well up to an inclusion level of 30%, compared with a fishmeal-based diet. The limitation was not protein function, but the high content of lipids in the insect-based meals, making higher inclusion rates nutritionally and technically unfeasible.

In the *ad hoc* digestibility study described in section 4.3 of this thesis, brewer's yeast, BSF meal and cricket meal were compared with fishmeal. Such an analysis has not been performed previously for Asian seabass diets. The data obtained are essential if the intention is to use these feed sources in practical diet formulation, as a sustainable alternative to soya and trash fish in the diet of Asian seabass.

5.2 Asian seabass culture, present state (Paper I)

The survey of smallholder fish farms and feed utilisation (Figures 1 and 2) showed that cage farming is more common than pond farming in coastal Cambodia. Cage culture in open water offers a low-investment opportunity for fishermen to change from catching wild fish to culture fish for sale (Sowles *et al*., 1994). However, culturing in open water poses an increased risk of transfer of diseases to both cultured and natural fish populations (Nash *et al*., 2001). Other risks with cage farming include fish escapes from cages that may affect wild fish populations via competition and potential interbreeding. One key issue for farmers with regard to both the environment and profitability is the feedstuffs used for farmed fish and the feeding regime. In Koh Kong province, use of commercial dry pellets is uncommon, while in Kampot and Preah Sihanoukvill dry pelleted feeds (combination with trash fish) are used by less than 20 % and more than 45 % of farms respectively. When farmers were asked about changing from feeding trash fish to cultured fish, the responses showed that between 20% and 40% of farmers want to change from feeding

trash fish to dry pellet since they understand the advantages of the latter. This is therefore a good starting point from which to explore alternative feedstuffs with acceptable quality and cost. Imported pelleted feed from Thailand and Vietnam for marine fish is expensive (2.3 USD kg^{-1}) compared with the cost of trash fish (0.2-0.3 USD kg^{-1}). Farmers who have medium-scale investments have the ability to buy pellets, but normally not for the whole culture period. Therefore they tend to use dry pellets only in the early stage of culture, when fish weigh between 1 and 2 g. In the grow-out stage (large fish), fresh trash fish is minced and used as feed directly in ponds or cages. The farmers need to expend time and labour to prepare the trash fish, a further disadvantage of this method. Consequently, if pelleted feeds could be produced on the farm or sourced locally, it would be more cost-effective for farmers to feed their fish with pellets. In Kampot province, more than 40% of farmers have some industry-relevant education in the form of attendance at workshops on culture techniques and homemade feeds, but not specific to marine fish, with most workshops covering freshwater fish. Some farmers believe that fish fed with the trash fish taste better. The reason may be that trash fish has a different protein, fat and mineral composition than dry pellet feeds (Haard, 1992). On the other hand, using dry pellets in cage culture can reduce feed waste into the water column around the fish farm compared with trash fish (Hansen *et al*., 1990). The small particle size of feed waste generated from trash fish leads to wider dispersion and a greater impact upon a larger area (Wu *et al*., 1994). Apart from settled waste feed, suspended solid waste and dissolved waste also have a higher nitrogen content because of the poor feed conversion of trash fish diets (Leung *et al*., 1996). Improved feed quality and access to high-quality, affordable, locally available dry feeds could therefore also reduce feed waste by producers in the aquaculture industry who are currently using trash fish.

5.3 Growth performance of fish (Papers II-IV)

Salinity varies in the diverse farming environments of coastal Cambodia. The trials in Paper II were conducted to examine the effect of different salinity levels on growth performance of different stages of Asian seabass (fry and fingerlings). These two life stages had not been compared previously with regard to salinity tolerance and the data derived for fry and fingerling were analysed separately. It was found that the salinity did not significantly affect the growth of the fish, so farmers with access to water of different salinity levels will not be disadvantaged in culture of Asian seabass fry or fingerlings. As performance of fry was unaffected within the salinity ranges tested, Asian seabass stock can be transported to farms as fry, which will provide a cost

saving to those who live far from fish production centres compared with shipping fingerlings.

Asian seabass is euryhaline (exhibiting wide salinity tolerance ranges). Otherwise, fish reared in water outside their optimal salinity ranges may increase energy allocation towards osmoregulation, to regulate within the 7-10 psu salinity range (Stickney, 1991). This means diverting energy away from other processes, such as activity, growth, and reproduction (Swanson, 1998). In the fry trial in Paper II, there was some mortality during the final period due to cannibalism, possibly because of the restricted feeding regime applied (10% of fish biomass). In Asian seabass culture, cannibalism can cause severe losses during the early stages of development, particularly before fish reach a length of about 10 cm (Qin *et al*., 2004). In the fingerling trial in Paper II, feed was provided to satiation by hand and continued until some feed was left uneaten, most likely explaining the relatively high FCR (>2) in the fingerling trial. There was no effect of salinity on condition factor of Asian seabass, in line with previous findings. In addition, there is no concern with the condition factor for fingerling with the value ranging from 1- 2% of standard (Nehemia *et al*., 2012).

Brewer's dried yeast was tested as a replacement feed ingredient for trash fish in diet for Asian seabass in Paper III because it contains high levels of nucleic acid nitrogen (mostly in the form of RNA), ranging from about 20 to 25 % of total protein (Knorr *et al*., 1979). In most monogastric mammals, high levels of dietary nucleic acids elevate plasma uric acid and produce toxicological effects (such as disturbances in the metabolism of fat, carbohydrate, and uracil) (Heft & Davies, 1976). In contrast, fish have the enzymatic capacity to metabolise uric acid into urea by uricase (Langeland *et al*., 2013) making fish an excellent target species for single-cell protein (such as yeast) as a protein source in their feed.

Asian seabass grew well on the yeast-based diet, with a slight tendency ($p>0.05$) over time for reduced growth in groups fed higher levels of yeast. This was most prominent in the hapas, while for fish kept in tanks this tendency was smaller, possibly because they had the opportunity to feed from the bottom of the tank, giving them a longer time to accept the feed. This would be consistent with a possible taste difference in yeast compared with fishmeal. No effect of diet was detected in condition factor of the fish, indicating a similar growth structure independent of the major protein source in the diet. Nehemia *et al*. (2012) suggest that a condition factor >1 in Asian seabass indicates good condition. This agrees with Oliva-Teles and Gonçalves (2011) and Ozorio *et al*. (2010), who report that brewer's yeast can replace up to 50% of fishmeal in the diet of European seabass (*Dicentrarchus labrax*) and

pacu (*Piaractus mesopotamicus*) with no effects on growth. Ebrahim and Abou-Seif (2008) observed reduced growth rate in Nile tilapia (*Oreochromis niloticus*) fed a higher level of brewer's yeast (0.3% supplementary glucan and 17.5% or 35% brewer's yeast) and suggested this to be an effect of low levels of methionine and sulphur-containing amino acid in the higher replacement diet. Brewer's yeast is considerably lower in methionine than fishmeal and soybean meal (Ebrahim & Abou-Seif, 2008), which could be the underlying cause for the tendency (although non-significant) for reduced growth in high yeast inclusion diets in Paper III.

Several studies, including those by Li and Gatlin (2003) working with striped bass (*Morone chrysops* × *M. saxatilis*) and Ortuño *et al*. (2002) working with gilthead seabream (*Sparus aurata*), report that dietary yeast improves the growth performance and the immune system. Robertsen (1999) observed that the activation of the immune system may involve ß-glucans from yeast cell wall stimulating phagocytic function and increasing the survival in several fish species after challenge with pathogenic bacteria (La Patra *et al*., 1998). Furthermore, glucan- and yeast-supplemented diets (0.3% glucan and 3.5% brewer's yeast) fed to tilapia have been found to result in significantly higher growth rate and lower feed conversion ratio than other test diets (Amin *et al*., 2015). In Paper III, fish mortality occurred in all treatments after day 30 in both hapas and tanks, indicating that the problem was more nutrition-based than due to health. Symptoms were similar to those described for nutrient deficiency in Asian seabass. Rumsey *et al*. (1991) reported that rainbow trout (*Oncorhyncus mykiss*) fed 50% and 75% inclusion of brewer's yeast in the diet took the feed pellets into their mouth and then expelled them. This rejection of feed suggests that some factors present or lacking in brewer's yeast reduce the acceptability of this feedstuff and limit feed intake. In Paper III, feed intake was lower in the fish receiving the diets 20% brewer's yeast compared with fish fed the 37 and 47% brewer's yeast diet. However, feed refusal was not observed in either hapas or tanks, perhaps because 47% brewer's yeast inclusion is much lower than in previous studies. In addition, although the weight gain was similar in fish fed brewer's yeast compared with the control, FCR was significantly higher ($p=0.54$ in hapas, $p=0.33$ in tanks) in fish fed diets with higher levels of yeast inclusion. This possibly reflects slightly lower digestibility of the yeast protein compared with fishmeal protein and is consistent with findings in the digestibility trial indicating apparent digestibility for brewer's yeast of 81.5% (Table 15), compared with close to 90% for most fishmeal. The 81.5% digestibility value is consistent with previous findings for baker's yeast in Arctic charr (*Salvelinus alpinus*) and Eurasian perch (*Perca fluviatilis*) (Langeland *et al*., 2016). It is also possible

that brewer's yeast contains anti-nutritional factors (*e.g*. nucleic acids, see above), which if present in high enough concentrations hamper the performance of monogastric animals, including fish. Studies by Huyben *et al*. (2018) and Schulz *et al*. (1976) indicate possible effects of the high nucleic acid content on erythrocytes, possibly as an effect of overload of the internal antioxidant system.

In the investigation of BSF larvae and cricket meal as alternative, sustainable, locally sourced ingredients for production of pelleted feeds, the fish were switched from a commercial floating dry pellet diet to sinking pellets in the experimental diet (Paper IV). Based on previous experience (Hamre *et al*., 2002), the fish were given ample time to adapt to this change, but a comparison between fish reared in parallel to the experimental fish and maintained on the floating diet indicated that the experimental diets were not competitive and that the fish grew less well on those pellets. This was independent of protein source, *i.e*. fishmeal-based or alternative ingredient. In this trial, the diet was formulated based on a final level of 45% of crude protein (CP) in each individual test diet and, when measured as crude protein content, this target was achieved in all diets. However, on complementing the gross analysis used to formulate the diets, major differences in non-protein nitrogen (NPN) were revealed. Adult insects contain high levels of chitin, which is the major component of their exoskeleton. This was the major reason for using BSF larvae instead of pre-pupae or pupae, where the chitin level is more prominent. In the case of cricket meal, this option is not possible as the nymph is a small copy of the adult animal. Surprisingly, the feed ingredient displaying the most pronounced discrepancy between crude protein and amino acid content was in fact the fishmeal, not the insect meal. In fact, the BSF meal consisted of nearly only protein nitrogen and the cricket meal had some NPN (21% of total CP), while the fishmeal only had 36% protein N, calculated as relative amino acid weight of the calculated crude protein, based on a 6.25 x N (Table 7). It was concluded that this low amino acid content in fishmeal protein must be the result of the meal being spiked with high nitrogen levels from a non-protein source, such as urea or melamine. This indicates that the common practice of only using gross chemical analysis as the basis for feed formulation should be complemented with methods able to discern between amino acid nitrogen and NPN, by using methods based on binding to amino acids (*e.g*. the Lowry, Bradford or bicinchoninic acid assay; Chang, 2010).

In spite of the discrepancies between crude protein and protein nitrogen, the pelleted test diets performed equally well. The higher FCR and low whole body crude protein content in fish fed the control diet could possibly be ascribed to the high content of NPN in the fishmeal in this diet. Overall, the

work in this thesis indicated that these alternative protein sources should be further evaluated as possible protein and lipid sources for Asian seabass diets, especially in low-resource environments such as Cambodia where they can solve the problems of waste valorisation and of replacement of non-sustainable feed ingredients like trash fish.

The proportion of cricket meal incorporated into the diets in this thesis was 147, 300 and 443 g kg^{-1} (Table 8). This is similar to levels employed in the studies by Taufek *et al*. (2016a, 2018), who likewise reported suitability of the ingredients at these levels. Similarly to single-cell protein, cricket meal has been reported to improve fish health in terms of resistance to *Aeromonas hydrophila* infection in African catfish (*Clarias gariepinus*) when the fish were fed a diet containing cricket meal at 350 and 400 g kg^{-1} instead of fishmeal. In addition, incorporating chitin into marine fish diets has been reported to enhance the innate immune system activity in sea bream (Esteban *et al*., 2001), stimulate macrophage activity in rainbow trout (Sakai *et al*., 1992), and increase growth rates and assimilation efficiencies in fish in general (Kono *et al*., 1987).

In Paper IV, it was found that high inclusion levels of BSF in the diet of Asian seabass reduced growth and increased FCR. Similar effects have been reported by Katya *et al*. (2017) in Asian seabass reared in fresh water, by Kroeckel *et al*. (2012) in channel catfish (*Ictalurus punctatus*), by Newton *et al*. (2005) in rainbow trout and by St-Hilaire *et al*. (2007[b]) in turbot (*Psetta maxima*). On the other hand, Bondari and Sheppard (1981) found no effect on body weight and total length in tilapia and channel catfish fed a control diet or a mixed diet consisting of 50% insect larvae and 50% commercial diet with high (45%) and low (30%) crude protein levels. Katya *et al*. (2017) performed studies on Asian seabass in freshwater environments and concluded that the maximal dietary inclusion level of protein from BSF larvae meal as a fishmeal protein replacer could be greater than 28.4%, but less than 50%, based on optimum growth performance and fish whole-body proximate and amino acid composition. However, Asian seabass may have limited efficiency to digest chitin, and thereby insect meal. Henry *et al*. (2015) assumed that the maximum dietary fishmeal replacement by BSF in aqua feed should range from 6 to 25 %, depending on the fish species. However, dietary supplementation with lysine and methionine allowed dietary BSF level up to a maximum of 25% when fed to Atlantic salmon (Lock *et al*., 2016). Other studies on rainbow trout have shown that the negative effect of high inclusion levels of BSF is substrate-dependent (Sealey *et al*., 2011). Tomberlin *et al*. (2002) observed that the development and various life-history traits of BSF are highly dependent on the growth substrate used. In Atlantic salmon, whether the

substrate is of marine origin or contains a high level of ingredients is reported to be important (Belghit *et al*., 2018). This is most interesting and indicates that diets including BSF have major potential, but that possible synergies with marine components, either in the substrate used to feed the larvae or in the final diet, must be identified in order to optimise this feed source. Possible carry-over effects of essential nutrients from marine sources such as seaweed can create a win-win situation where BSF larvae include beneficial marine components, which can otherwise not be included at high concentrations in feed for carnivorous fish, due to the high content of complex carbohydrates in the native source (de Jesus Raposo *et al*., 2015). For example, including brown algae (*Ascophyllum nodosum*) in BSF culture media has been found to improve the nutritional composition of the BSF larvae by introducing marine nutrients such as the poly-unsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), iodine and vitamin E (Liland *et al*., 2017).

The values of hepato-somatic index (HSI) and viscero-somatic index (VSI) found in this thesis (Table 14) were lower those reported by Belghit *et al*. (2018). No significant differences in HSI and VSI were found between the treatments (range 1.12-1.61% and 5.40-6.48%, respectively) (Table 14). The value of HSI provides an indication of dietary effects on liver function. The liver is a key organ for metabolism and the values of HSI detected in Paper IV did not exceed the standard range (1-2%) shown to be associated with a functional feed (Dernekbaşi, 2012). Espe *et al*. (2012) speculated that the low level of taurine in BSF may affect the fat content of fish liver, as the addition of taurine to a high-plant diet (low in taurine) had a positive effect on lipid metabolism and reduced liver lipid depositions in juvenile Atlantic salmon. However, replacement of fishmeal with BSF did not affect the HSI when insect meal was included in the diet of Atlantic salmon at a maximum level of 150 g kg^{-1} diet (Espe *et al*., 2012). However, Belghit *et al*. (2018) used 600 g BSF kg^{-1} diet in combination with insect oil in the diet of freshwater salmon, which could explain the difference in results between these two studies.

5.4 Digestibility study (this thesis)

Digestibility has been defined as the amount or proportion of nutrients, or categories of nutrients such as crude protein, that disappears from a meal as it passes through the digestive system and is excreted in faeces (NRC, 2011). In practice, digestibility is primarily a measure of disappearance of nutrients from the ingested feed. Diet design, feeding strategy, faeces collection method and calculation method are all parameters affecting the apparent digestibility coefficient (Glencross *et al*., 2007). Thus, the digestibility of the diet has been identified as one of the key factors in evaluation of prospective novel feed

ingredients to be included in aquaculture (Glencross *et al.*, 2007). However, during the work in this thesis little was known about the nutrient digestibility of cricket meal, BSF meal and brewer's yeast as ingredients in formulated feed for Asian seabass. Therefore, a small *ad hoc* study was conducted, in association with the trial in Paper IV, to assess the nutrient digestibility of all diets used in thesis, *i.e*. those including brewer's yeast, fishmeal, BSF larvae meal and cricket meal.

In the digestibility study, crude fibre was used as an inert marker (Tacon & Rodrigues, 1984). The digestibility values obtained for cricket meal were lower those in Taufek *et al*. (2016b), who report apparent digestibility coefficients (ADC) of 0.81 and 0.898 for crude protein and energy, respectively, compared with 0.634 and 0.739, respectively, in this thesis (Table 15). However, their value for energy digestibility (64.4%) was lower than the value obtained in this thesis (71.4%) (Table 15). There were significant differences ($p<0.001$) in the ADC of crude protein in the test diets, which was 0.55, 0.63, 0.81 and 0.82 for the BSF meal, cricket meal, brewer's yeast and control diets, respectively (Table 15). These values are considerably lower than the range in digestibility values typically observed for fishmeal (86-97%) (NRC, 2011). The digestibility of fishmeal diet should be higher than that of insect meal, since the cuticle of insects contains chitin that is linked to protein, reducing the apparent and true digestibility of nitrogen. Chitinase genes have been sequenced in several carnivorous marine teleosts, confirming that some fish are able to produce chitinase and thus to degrade chitin (Kurokawa *et al*., 2004). However, Asian seabass was not included in that study and its capacity in this respect remains unknown. The value obtained for BSF crude protein digestibility (55.2%) (Table 15) was much lower than the 77.7% found by Bosch *et al.* (2014). This is still lower than that normally accepted for fishmeal and is most likely due to the chitin content. On the other hand, as discussed above, BSF could provide other macro- and micro-nutrients, compensating for the lower digestibility. Similarly, Renna *et al*. (2017) observed a significant reduction in protein digestibility of rainbow trout when fish meal was replaced by 50% BSF but concluded that, in spite of reduced digestibility, dietary fishmeal can be replaced by partly defatted BSF larvae meal up to 50% of substitution (40% of inclusion in diet), without negative effects on growth performance, condition factor, somatic indices, physical quality parameters or gut morphology. Taken together, the results presented in this thesis call for further investigation of insect meals and yeast as alternative, sustainable and affordable locally sourced ingredients for Asian seabass diets in Cambodia.

6 General conclusions

- A high percentage of seabass farmers in coastal Cambodia are willing to switch from trash fish as feed if suitable and affordable alternatives become available
- Asian seabass juvenile and fingerlings performed equally well independent of salinity level, but with a slight increase in growth efficiency at isotone salinities
- Locally obtained brewer's yeast, a by-product from beer brewing, has high potential to function as an alternative to fishmeal in the diet of Asian seabass, given some adjustments
- Locally produced full-fat protein meal from insects, using local farm waste as substrate, has high potential as a protein and lipid source in Asian seabass diets

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Popular science summary

This thesis addresses development of marine aquaculture in Cambodia in general and the problem of non-sustainable use of trash fish as feed to Asian seabass in particular. Cambodia has one of the highest rates of fish consumption per capita globally (up to 80 kg per person and year). Fish thereby constitute a major protein resource with special importance for the resource-poor part of the population. At present, major development for hydropower is ongoing in the Mekong river, threatening the spawning migration of most of the main wild food fish species of Cambodia. Development of sustainable aquaculture is therefore of national interest in Cambodia. However, the fish farming industry is in major need of improved and environmentally friendly farming practices. The industry is also currently dependent on imported commercial feeds based on soy and fish ingredients, which are expensive and also difficult to obtain, *i.e*. limited market access. This has prompted many farmers to use locally obtained trash fish, often harvested from threatened populations. The low hygiene and risk of oxidation of these products introduces a risk of toxins and pathogens to the farmed fish.

A survey in this thesis determined the state-of-the-art in Cambodian Asian seabass farming, including use of commercial diets, trash fish, location and production volume/system in the different marine regions of Cambodia. A study was then carried out to investigate whether farming environment in terms of salinity levels is a major factor in Asian seabass production. This information is vital in the formulation of a national strategy, as the survey showed that fish farming is currently carried out in marine, brackish and freshwater locations. Asian seabass is a euryhaline species, tolerating a wide variety of salinities. The study showed a slight preference for isotone salinity, but indicated that all salinity levels tested functioned adequately for fish farming. In addition, Asian seabass fry and fingerlings performed equally well, independent of salinity, so farmers could re-stock using fry instead of fingerlings, reducing the cost.

The survey revealed that a high percentage of local farmers are willing to switch from trash fish as feed if suitable and affordable alternatives will become available. Therefore studies were carried out on potential alternative feed ingredients. One such study examined the possibility of using spent brewer's yeast as a local industrial by-product from the brewing industry and well-controlled source of low-cost protein for Asian seabass diets. The study was conducted both in hapas (small cages) and in tank environments. Irrespective of farming environment, brewer's yeast showed positive results as a partial replacement for protein from fish and soy. Thus locally obtained brewer's yeast has good potential to function as an alternative to fishmeal in the diet of Asian seabass, given some adjustments.

In another study, two types of insects, local crickets and black soldier fly (BSF) larvae, were tested as complementary, low-cost sources of protein and lipids in the diet of Asian seabass. Both these insects were shown to grown well on different waste streams available in Cambodia, *e.g.* cassava tops, food/fish waste *etc*. Both insects also showed good potential to be produced in large- or small-scale production units. In general, both sources functioned well as a protein source up to an inclusion level of 30% compared to a fishmeal-based diet. Thus full-fat protein meal made from locally produced insects reared using local farm wastes as substrate has good potential as a protein and lipid source in Asian seabass diets.

Digestibility studies were performed on the brewer's yeast, BSF meal and cricket meal, and the results were compared with those for fishmeal. Such an analysis has not been performed previously in Asian sea bass, but is essential if these sources are to be used in practical diet formulation as a sustainable alternative to soya and trash fish in the diet Asian seabass. The results revealed acceptable digestibility of crude protein, lipids and energy in the alternative feedstuffs. However, they also revealed possible illegal spiking of other feed ingredients with non-protein nitrogen (NPN). Therefore monitoring and probably also control mechanisms are necessary to detect spiking with NPN when using the alternative feed sources described in this thesis.

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